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Kenneth Bruce Davis Jr
Louisiana State University and Agricultural & Mechanical College

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SEASONAL AND DIURNAL VARIATIONS OF SODIUM, POTASSIUM, AND CHLORIDE LEVELS IN THE PLASMA AND BRAIN OF THE MIGRATORY WHITE-THROATED SPARROW, ZONOTRICHIA ALBICOLLIS.

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1970
Physiology

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Seasonal and Diurnal Variations of Sodium, Potassium, and Chloride Levels in the Plasma and Brain of the Migratory White-throated Sparrow, *Zonotrichia albicollis*

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Zoology and Physiology

by
Kenneth Bruce Davis, Jr.
B. A., University of Arkansas, 1963
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January, 1970

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Abstract

Studies of hormones thought to be involved in bird migration have indicated that the diurnal timing of the levels of the hormones is important in regulating physiological and behavioral events in the birds annual cycle. Further, hormones thought to be involved in the control of nocturnal locomotor activity of bird migration have been shown to be effective in increasing nervous sensitivity, intracellular brain sodium, and locomotor activity in mammals.

This study was done to determine whether seasonal and diurnal rhythms of blood and brain electrolytes exist in the migratory white-throated sparrow, and to ascertain whether the rhythms reflect altered physiological states at different times of the year. A diurnal rhythm of extracellular/intracellular sodium concentration of the brain might reflect altered nervous sensitivity which may be correlated with seasonal changes in locomotor activity of the white-throated sparrow, particularly that of nocturnal locomotor activity.

Plasma concentration and brain content of sodium, potassium, and chloride were measured at several times of the day on May 15 (spring migration), and August 7 (postnuptial molt). Brain electrolyte contents were

also measured on April 5 (prenuptial molt), and plasma potassium levels were measured in April and on May 5. Sodium and potassium were determined by flame photometry and chloride was determined with an automatic chloride titrator. The diurnal rhythms of extracellular/intracellular brain sodium ratio were calculated for mid-May and August.

A unimodal diurnal rhythm of brain sodium content was present in April and mid-May. A similar rhythm of brain potassium was present in mid-May. In April a rhythm of brain potassium content similar to that in May appeared to be present but was not statistically verified. The phase angles of the brain electrolyte rhythms in April and mid-May were similar in their relation to the photoperiod. In August no rhythm of brain sodium was apparent and a bimodal rhythm of brain potassium occurred. No diurnal rhythm of brain chloride was present in mid-May or August. In April a small, but significant peak of brain chloride was present at sunset. In mid-May and August a diurnal rhythm of plasma sodium was present. In August the amplitude of the rhythm was lower and the time of the peak was shifted by eighteen hours. A diurnal rhythm of plasma potassium occurred in April, early May, mid-May, and August. The mid-May peak was also shifted from that in August by eighteen hours.

A diurnal rhythm of the brain sodium ratio occurred in mid-May but none was present in August. In mid-May the brain sodium ratio was highest at sunrise when locomotor activity was lowest. The brain sodium ratio decreased during the day and remained low until midnight when nocturnal locomotor activity was occurring. A low brain sodium ratio has been correlated with increased nervous sensitivity in mammals. Some aspects of locomotor activity in mid-May might be due to a change in the sensitivity of the nervous system. No correlation of locomotor activity with brain sodium ratio was present in August.

Seasonal comparisons using the diurnal mean indicated few quantitative differences in seasonal electrolyte content. Blood chloride and brain potassium were lower, and blood sodium was higher in mid-May than in any other season. The most conspicuous seasonal differences of most of the measurements were in phase angle shifting and amplitude of the diurnal rhythms.

Possible endocrine controlling mechanisms of electrolyte diurnal and seasonal rhythms are discussed. A phase angle synergism between corticosterone and prolactin was suggested for causing the presence of the diurnal rhythm of the brain sodium ratio in mid-May.

Introduction

Levels and distribution of inorganic ions in the body fluids of vertebrates have profound effects on all the body cells. Regulation of these ions, particularly sodium, potassium, and chloride, affect body water, osmotic pressure, and membrane potential, and thereby affect the movement of many solute particles. One of the most important effects of the distribution of these ions is the establishment and maintenance of electrical potentials across cell membranes. The membrane potential plays an important role in the function of muscles and nerves. High sodium or low potassium concentrations on the outside of cells tend to increase the resting potential. The membrane potential may become so high that the nerves can no longer transmit impulses to the muscles. High levels of potassium in the extracellular fluid have a depressor effect on conduction of nerve and muscle impulses along membranes due to a decreased resting potential.

Variations in the sodium, potassium, and chloride levels of plasma have been correlated with various behavioral and physiological changes in vertebrates (for review see Eiduson et al., 1964; Hoagland, 1954; Lobban, 1960; Lewis and Lobban, 1957 a, b). It has been suggested that the regulation of sodium between the

extra- and intracellular compartments of the nervous system affects the sensitivity of the nervous system (Woodbury et al., 1957). Changes in the concentration of intracellular brain sodium and/or the ratio of extracellular/intracellular brain sodium concentration (brain sodium ratio) are associated with changes in brain excitability as measured by electroshock seizure threshold (EST) in the rat. Decreased brain sodium ratio correlates with increased brain excitability demonstrated by a decrease in EST, whereas increased brain sodium ratio correlates with decreased brain excitability. No correlation was found between nervous sensitivity and brain or plasma levels of potassium. Normal cells are generally saturated with potassium when the extracellular concentration of potassium is above 2 mEq/liter, and the action potential is thought to result mostly, if not entirely, from sodium flux.

Diurnal variations of sodium, potassium, and chloride concentrations in both the plasma and urine have been studied in man on normal twenty-four hour (light-dark) cycles and on experimental cycles of different lengths (for review see Mills, 1966). Consistent rhythms of sodium and chloride excretion were found to shift rather quickly in accordance with experimental time routines, whereas potassium was much more resistant to

change and tended to retain its twenty-four hour rhythmicity (Lewis and Lobban, 1957 a, b; Lobban, 1960). Excretion of sodium, potassium, and chloride appear to be regulated by an internal mechanism and not to be directly controlled by habit (Mills, 1951). The diurnal rhythms of plasma electrolyties in man are usually irregular, notched, or biphasic, and always of very low amplitude (Mills and Stanbury , 1955; Wesson, 1964; Mills, 1966). However a distinct rhythm of plasma chloride has recently been found in fish (Meier, personal communication).

Adrenal and thyroid function have been correlated with changes in the sensitivity of the nervous system, both in the brain and in sense organs. Nervous disorders in humans are often associated with adrenal disorders and with the distribution of sodium and potassium (Eiduson et al., 1964). A higher than normal sensitivity to taste, smell, and hearing exists in patients with adrenocortical insufficiency. This sensitivity could be returned to near-normal levels by administration of carbohydrate-active steroids, but sodium retaining steroids did not alter the sensitivity levels (Henkin et al., 1962; Henkin and Bartter, 1966; Henkin et al., 1967). Further, axonal conduction velocity increased in patients with adrenocortical insufficiency (Henkin

et al., 1963), and conduction across the myoneural junction and latency measured by visually evoked responses was significantly delayed. These events were also corrected by carbohydrate-active steroids. Glucocorticoids apparently had opposite effects on axonal conduction and synaptic transmission. Synaptic transmission appeared to be facilitated while axonal conduction was slowed by carbohydrate-active steroids. Sodium-retaining steroids had little effect on either. The sensitivity of polysynaptic responses of the nervous system appears to be increased by carbohydrate-active steroids which results in greater overall nervous sensitivity.

Increased behavioral alertness and electroencephalograph (EEG) activity in rabbits follow injections of dexamethasone, a synthetic glucocorticoid (Shimada, 1966). Corticosterone and cortisol were found to be concentrated in the tissues of the central and peripheral nervous system of the cat and human (Touchstone et al., 1966). Both steroids were drastically reduced after adrenalectomy (Henkin et al., 1968).

Decreased steroid output in Addison's disease causes abnormally slow EEG waves (Engel and Margolin, 1941, Engel and Margolin, 1942). A similar slowing of the EEG pattern has been found in adrenalectomized rats (Bergen, 1951).

Cortisone restores the slow activity to normal (Thorn, 1949).

Deviations from normal blood electrolyte levels are often present in patients with untreated insufficient adrenal secretion. These patients often exhibit personality alterations, particularly negativism, depression, and irritability. Mental disturbances may occur independently of blood electrolyte disturbances and often may be corrected by cortisone (Cleghorn and Pattee, 1954). Perhaps the electrolyte disturbances may not be evident in the blood, but in the extra-intracellular distribution. Woodbury found no blood electrolyte changes correlated with changes in EST.

A number of adrenal steroids have been tested for activity in affecting the sensitivity of the nervous system in rats as measured by EST (Davenport, 1949; Woodbury and Davenport, 1949; Timiras et al., 1954; Woodbury et al., 1957). A rise in EST was interpreted as a decrease in nervous sensitivity, and a fall in EST was interpreted as an increase in nervous sensitivity. Deoxycorticosterone acetate (DCA) was the most effective in increasing EST in intact and adrenalectomized animals. Animals treated with DCA and whose EST was elevated were also found to have an increased brain

sodium ratio, and increased brain content of glutamic and aspartic acid. Animals in which EST was lowered had a decreased brain sodium ratio, glutamic and aspartic acid, and increased glutamine and asparagine in the brain. Cortisone and cortisol were both effective in decreasing EST.

After adrenalectomy the intracellular brain sodium concentration increased and brain excitability as measured by EST was higher. Brain excitability could be returned to normal by providing sodium chloride in drinking solutions. Apparently sodium chloride protected against the increase in brain excitability (Timiras et al., 1954; Woodbury, 1954). However adrenalectomy has been shown to decrease the spontaneous running activity and to enhance the susceptibility to audiogenic seizures (Griffith, 1949). Running activity was increased by cortisone or adrenocortical extract (both of which decrease EST). A decreased susceptibility to audiogenic seizures occurred when the rats were given large doses of sodium chloride. Locomotor activity of birds was increased by providing drinking water high in sodium chloride (Dawson et al., 1965).

These data suggest that the adrenal secretions responsible for increasing locomotor activity may promote

an increase in nervous sensitivity, as measured by EST or EEG, and a decrease in sensitivity of sensory systems, specifically those of taste, olfaction, and audition. Sodium chloride protects against decreased EST or sound-induced seizures in adrenalectomized rats and also increases locomotor activity.

The thyroid has been implicated in the regulation of brain excitability as measured by EST. Injections of thyroxine (T_4) and triiodothyronine (T_3) in rats increased brain excitability, increased intracellular brain sodium concentration, and decreased brain sodium ratio (Timiras et al., 1955). Thyroxine was less effective in adrenalectomized animals, and even less effective in adrenalectomized rats maintained on adrenocortical extract. He suggested that T_4 and T_3 injections result in increased adrenal activity as measured by glandular hypertrophy with very high doses and by increased ascorbic acid depletion in lower doses (Timiras and Woodbury, 1955). T_3 was more effective than T_4, perhaps because it enters the cell more readily. A similar relationship has also been indicated in birds. Atrophy in pigeon adrenocortical tissue occurs after treatment with thiouracil (Bhattacharya and Ghosh, 1963). Thyroxine in low doses caused increased adrenal weight whereas high doses caused adrenal atrophy (Miller and Riddle, 1942).

Fregly and Taylor (1964) have shown that T_4 conserves body sodium and water. Rats treated with propylthiouracil had excessive loss of sodium and water. The sodium and water content could be returned to normal values by administration of T_4.

Nocturnal restlessness of the migratory white-crowned sparrow has been induced by a synergism between prolactin and adrenocortical extract (Meier et al., 1965). Thyroxine has also been shown to heighten the amount of nocturnal restlessness in birds (Schildmacher, 1952). Merkel (1938) was able to increase migratory restlessness in fat birds with low doses of T_4 and to decrease activity with high doses of the same substance. However, nocturnal restlessness could not be induced in birds without a heavy deposit of fat. Premigratory fattening has been induced in photosensitive and photorefractory birds with prolactin (Meier and Farner, 1964; Meier and Davis, 1966).

During the nonmigratory period the white-throated sparrow exhibits locomotor activity only during daylight hours. During the migratory period, which occurs twice a year, the sparrows are active at night. The spring migration is a response to the increasing day lengths. Therefore, nocturnal migrants are excellent animals to study whether all locomotor activity is controlled by

the same mechanism or whether nocturnal activity and diurnal activity have different controlling mechanisms. A shift in the diurnal rhythm of activity of the adrenal, thyroid, pituitary, or all three might result in nocturnal locomotor activity in a normally diurnal bird. The mechanism of action of the elements responsible for nocturnal locomotor activity may result from an increased sensitivity of the nervous system due to a seasonal shift in the diurnal extra-intracellular movements of sodium ions.

Seasonal studies indicate that adrenal glands also have an annual rhythm of activity. Studies of several species of birds, measured histologically, indicate increased adrenal activity during the breeding season in nonmigratory birds (Burger, 1938; Raitt, 1968) and decreased adrenal activity during the breeding season in migratory birds (Lorenzen and Farner, 1964).

Direct measurements of plasma adrenocortical hormone at different times of the year are few. Seasonal variations have been reported in the Pacific salmon (Robertson et al., 1961), the duck (Assenmacher and Boisson, 1968) and the chicken (Resko et al., 1964). These studies did not consider diurnal variations of the various seasons. A shift in the phase of the diurnal rhythm at different times of the year could result in

obscuring or amplifying annual rhythms. Information on seasonal phase shifting of diurnal rhythms is sparse. Three studies which have considered the diurnal rhythm have shown that seasonal phase shifting occurs. In the white-throated sparrow the diurnal rhythm of pituitary prolactin (Meier et al., 1969) and plasma corticosterone exhibit different phase angles at different times of the year. The time of peak water and potassium excretion in man also shifts with the season (Ghata and Reinberg, 1954).

This study was done to determine the seasonal and diurnal levels of brain and plasma sodium, potassium, and chloride, and to ascertain whether the diurnal extra-intracellular movement of brain sodium is correlated with the seasonal and/or diurnal changes of locomotor activity. The phase angle of the rhythm in relation to the season and to the photoperiod was of particular interest. Measurements of these elements were taken during three times of the year in order to determine the phase angle of these parameters with respect to the photoperiod and to behavioral and physiological states of the birds in the three seasons. It was hoped knowledge of the seasonal diurnal relationships of the electrolytes might lead to a better understanding of the regulation of locomotor activity, particularly that of nocturnal restlessness in a migratory sparrow.

Methods and Materials

White-throated sparrows are small seed-eating, intermediate range migrants which are limited to North America. They migrate between their breeding grounds in northeastern United States and Canada and their wintering grounds which extend from New England to the Gulf Coast and northeast Mexico. They are easily accessible and do well in captivity. The birds used in this study were collected by trapping and netting from wintering flocks near Baton Rouge, Louisiana, and kept in an outdoor aviary for at least two months before being used. They were fed a mixture of chick starter mash throughout.

This bird was studied in April, early-May, mid-May, and August for several reasons. First, the birds exhibit a number of physiological differences in these months. In April wild birds are still on their wintering grounds. There is little body fat, the gonads are small, there is no nocturnal locomotor activity, and feathers are being replaced during prenuptial molt. They are photosensitive; however, the photoperiod is not yet long enough to initiate the events which precede migration. In May the white-throated sparrow deposits fat in preparation for migration, the gonads begin to recrudesce, and nocturnal restlessness occurs. The

birds respond with these events to a lengthening photoperiod (Rowan, 1932) and are therefore termed photosensitive. In August the breeding season is over, the gonads have regressed, there is little body fat, and postnuptial molt occurs. Although the photoperiod is still long, the birds no longer respond to it, and are termed photorefractory. The second important consideration was the comparative lengths of the photoperiod. In May the photoperiod is an hour and five minutes longer than in April; however, the May photoperiod is only fourteen minutes longer than in August.

The diurnal locomotor activity of several birds was monitored for five days preceding sampling. Activity was measured with an Esterline-Angus recorder, which was connected to a microswitch on the cage perch. The activity index was determined as the number of two minute intervals per hour with three or more hops.

Samples were taken from groups of birds every six hours beginning at sunrise and continuing for twenty-four hours on May 6, 1967, August 7, 1967, April 5, 1968, May 15, 1968, and August 7, 1968. Two additional groups were killed in May of 1968, nine and fifteen hours after the beginning of the photoperiod. Sunrise was determined from a sunrise-sunset table prepared by the Nautical Almanac Office, United States Naval

Observatory, Washington, D. C. In order to simplify presentation of the data, the values for each of the sampling times were adjusted to the beginning of the photoperiod. S was used to signify sunrise, and the other times were reported as S+ the number of hours after sunrise.

Birds were sacrificed in April during prenuptial molt. The birds killed on May 6, 1967 were gaining fat weight but exhibited no nocturnal locomotor activity. The birds sacrificed on May 15, 1968 were physiologically prepared for migration as determined by visual inspection of the large stores of subcutaneous fat and by the presence of locomotor activity at night. Nightly locomotor activity of caged migratory birds has been used extensively as an index of readiness to migrate (Rowan, 1932; Farner et al., 1954). The photorefractory condition of the birds sampled in August was evident by the appearance of postnuptial molt, low body fat, and by the absence of nocturnal locomotor activity.

Blood samples were taken by heart puncture, centrifuged, and the plasma sealed in capillary tubes and frozen for later analysis. Chloride levels were determined from 10 μl of plasma with a Buchler-Cotlove automatic titrator and reported as mEq/liter of plasma. For sodium and potassium analysis, 50 μl of plasma were

diluted in 5 ml of protein precipitant (5% trichloro-acetic acid, 10% iscpropyl alcohol in distilled water) and centrifuged. The supernate was then measured by flame photometry with a Beckman D U spectrophotometer equipped with a flame attachment. Sodium levels were determined first, and the potassium standards were prepared containing the average sodium concentration to blank out sodium interference. Sodium and potassium levels were also reported as mEq/liter of plasma. Only plasma potassium levels were determined on samples collected in May and August of 1967, and plasma sodium and chloride levels were not measured in April.

At autopsy the carcasses were weighed, and the gonads and whole brains were removed and weighed. The fat content of the body was determined by Soxhlet extraction with petroleum ether. Fat content of the birds was converted to percent lipid of the total dry weight.

Whole brains were dried in a vacuum oven, weighed, and ground up. The fat was extracted with three changes of petroleum ether at twenty-four hours per change, redried, and weighed. The fat-free dried brains were extracted with 10 ml of 0.1 N nitric acid for forty-eight hours. Chloride concentration of the extract was determined with the titrator. Sodium and potassium

concentrations were measured by flame photometry. Brain sodium, potassium, and chloride content were presented in two ways: (1) as mEq/kg of fat-free fresh brain weight according to the formula:

$$\frac{(10\ ml)\ (mEq/ml)}{G_f} \quad X \quad 1000 = \text{mEq/kg fat-free fresh brain weight}$$

10 = number of ml of the extract

G_f = fat-free fresh brain weight in grams

mEq/ml = concentration of the extract

and (2) mEq/kg fresh brain weight by substituting G_w (wet brain weight) for G_f in the preceding formula. Calculation of the extra-intracellular water and sodium content of the brain on a fat-free fresh weight basis was done by the method of chloride space described by Cotlove et al., 1951. The calculation based on fresh brain weight is a modification of Cotlove's method used by Timiras et al. (1955), and was done as a comparison. The intracellular sodium concentration was determined from the amount of intracellular water per kg brain weight, and the intracellular sodium content by the following ratio:

$$\frac{(Na)_c}{(H_2O)_c} = \frac{X}{1000}$$

$(Na)_c$ = intracellular brain content in mEq/kg brain weight

$(H_2O)_c$ = intracellular water in gm/kg brain weight

In order to minimize errors in the mathematical treatment of the data, means were not used in the calculations, but rather the data from each bird was calculated individually. Means for derived data were determined from the data from each individual in the group.

This method of determining extracellular space has been shown to give comparable results to measurements made by sucrose and inulin determination of intracellular water (Cotlove, 1954). It had the further advantage of not requiring an injection of the birds before sacrifice, a procedure which may have upset the concentrations and/or the rhythms of the parameters measured.

Extracellular concentrations of potassium are so small compared to the intracellular levels, that brain potassium content was considered as completely intracellular. Intracellular potassium concentrations could then be calculated from the total potassium and the intracellular water content.

The significance of differences between seasonal means was determined by Student's "t" test. Statistical evaluation of the diurnal variations of water, sodium, potassium, and chloride was determined by a one-way analysis of variance. Those diurnal measurements which

had F values of sufficient magnitude indicating a significant difference between means were treated further by Duncan's Multiple Range test (Steel and Torrie, 1960) at the 95% confidence interval. The peak value of each of the diurnal variations was tested against the trough value by Student's "t" test for a higher degree of significance (99%).

Results

Comparison of the behavioral and physiological states of white-throated sparrows in April, early May, mid-May, and August

The means of the various parameters used to verify the different physiological conditions of the birds at the various times of the year are shown in Table 1. Body weight, fat level, gonad weight, and oviduct weight were all much higher in mid-May than in April or August. Although the data from early May are incomplete, the body weight and fat percent of the body at this time is higher than the values in April and August, but not as high as those found in mid-May. No evidence of molt was observed in early or mid-May. Molting was observed in both April and August. Prenuptial molt was present in April and postnuptial molt was present in August.

The diurnal activity pattern which occurred in the various seasons is presented in Figure 1. In April, early May, and August, the activity pattern was very similar. No locomotor activity occurred at night and there were two peaks of daylight activity, one at sunrise and another at sunset. During mid-May, nocturnal locomotor activity, or Zugunruhe, was evident beginning immediately after sunset and lasting until about 0030 hours. Daylight activity consisted of a morning activity

period which reached a peak about three hours after the beginning of the photoperiod, then fell to a constant level until another sharp drop occurred shortly before and lasting until shortly after sunset.

There were three main differences in the locomotor activity pattern between mid-May and the other seasons. The mid-May pattern differed in the following ways: (1) the absence of an activity peak at sunset (2) presence of locomotor activity at night, and (3) a morning activity peak occurring three hours after sunrise rather than immediately after sunrise.

Brain sodium, potassium, chloride, and water content

The mean diurnal levels of total brain sodium, potassium, chloride, and water in April, mid-May, and August are shown in Tables II and III. The values in Table II were calculated as mEq/kg fat-free fresh brain weight, and those in Table XVI represent mEq/kg fresh brain weight. The electrolyte values from Table II are presented in Figures 2., and 3. to show better their relationship to the photoperiod.

No distinct diurnal rhythm of brain chloride content was apparent in any of the three months (TablesVII, X, and XIV). The overall chloride levels in April and mid-May were similar and both were significantly lower than the chloride levels observed in August.

A diurnal fluctuation of brain sodium occurred in April and mid-May as indicated by an analysis of variance tested against the photoperiod (Tables IV and VIII). The peak of brain sodium content occurred at S+9 in mid-May and at S+12 in April and the trough at S in both months. On closer inspection the April and mid-May rhythms are similar. If the additional sample at S+9 in mid-May had not been taken, the shape of the curve would have been indistinguishable from the April curve. No diurnal fluctuation of brain sodium content in August was apparent (Table XII). There were no differences in the overall seasonal levels of brain sodium content.

Diurnal fluctuations of brain potassium content were found in mid-May, and August. A different pattern, however was observed between the mid-May rhythm and the August rhythm. The pattern observed in April was similar to that in mid-May but was not significant (Table V). In mid-May and August the diurnal variation was verified statistically (Tables IX and XIII). The peak in mid-May occurred at S+12 and the trough at S+6. The dip at S+15 in mid-May does not represent a true second trough in as much as the mean value at this time is not significantly different from the values observed at S+12 and S+18. In August two peaks of brain potassium

content were observed. These peaks occurred twelve hours apart, one at S+6 and the other at S+18.

There was also a seasonal difference in brain potassium content. In mid-May the overall brain potassium content was statistically lower than in April or August; furthermore the level observed in August was lower than that in April.

No apparent diurnal variations in brain water content occurred in either April or August (Tables VII and XV). The diurnal levels of total brain water did fluctuate in mid-May (Table XI). The greatest amount of brain water was observed at S+6 and the least amount at S+12. Water content of the brain in mid-May and August was practically identical and both were significantly higher than the water content of the brain in April.

The values for brain content of water and electrolytes compare closely with reports for these levels in rats. Values for these substances are reported as g/kg or mEq/kg of fresh weight by many investigators (Woolley and Timiras, 1964; Timiras et al., 1955; Woodbury, 1955). The values given here in Table III are calculated on a fresh weight basis to facilitate comparisons with those in the literature.

Brain content of sodium, potassium, and chloride were reported both as mEq/kg fat-free fresh weight and

as mEq/kg fresh brain weight to point out differences that are present dependent on how the data are analyzed. Values based on fat-free fresh weight were about 2.5 mEq/kg greater than values based on fresh weight. Fat-free fresh weight should be a more realistic way to report tissue electrolyte content since these ions are not soluble in fat and are therefore only distributed in the water phase. This would be especially important in determining extra and intracellular water content. The method of chloride space for an estimation of extracellular water content, published by Cotlove (1951), was done on the basis of fat-free fresh weight of muscle tissue. However, extra-intracellular ionic movements in the brain and ion content of the brain have been done on the basis of fresh weight by many investigators (Davenport, 1949; Woodbury and Davenport, 1949; Timiras et al., 1955; Woodbury, 1955; Woodbury, 1957; Woolley and Timiras, 1964; Woodward et al., 1967).

Plasma sodium, potassium, and chloride

Sodium and chloride concentrations of plasma collected in mid-May and August are given in Table XVI. The data are presented as mEq/liter plasma. No diurnal fluctuation of chloride concentration was apparent in

either month (Tables XVIII and XX). The overall mean in August was higher (p= .01) than the overall mean observed in mid-May.

A unimodal rhythm of plasma sodium was found in both mid-May and August (Tables XVII and XIX), but the shapes of the variations with respect to the photoperiod, as well as the amplitude, were different. In mid-May plasma sodium concentration was highest at S, and lowest at S+18. The amplitude of the mid-May rhythm was about 16 mEq/liter. In August, plasma sodium concentration reached a peak at S+18, and the trough occurred at S. Further, the amplitude of the August rhythm was only 7 mEq/liter, or about one-half of the amplitude found in mid-May. The overall average of plasma sodium concentration in mid-May was higher than that found in August (p= .01). Plasma sodium rhythms in mid-May and August are presented in Figure 4.

The seasonal values reported here for sodium (mid-May: 175.8; August: 170.3) and chloride (mid-May: 119.9; August: 127.5) concentrations are higher than values reported for many vertebrates including man, rat, dog, chicken (Prosser and Brown, 1962) and white-winged and inca doves (MacMillen and Trost, 1966). Sodium concentrations in the plasma of the white-throated sparrow appear to be similar to sodium levels

found in the mourning dove (Smyth and Bartholomew, 1966), house finch (Poulson and Bartholomew, 1962), and the savanah sparrow (Poulson and Bartholomew, 1962). The birds in all these studies were captured during the winter and held on an artificial 12-12 LD photoperiod.

Plasma chloride concentrations in white-throated sparrows were similar to values of 135.9 in the mourning dove (Smyth and Bartholomew, 1966) and 130 in the house finch (Poulson and Bartholomew, 1962). No references were found for plasma sodium or chloride concentration of migratory birds during the migratory season.

The diurnal levels of plasma potassium determined in April, early May, mid-May, and August are presented in Table XXI. These data are also reported as mEq/liter plasma. Diurnal rhythms were present in all four months (Tables XXII, XXIII, XXIV, and XXV).

In April the peak of plasma potassium occurred at S+12, and the trough at S. Different patterns of diurnal variation of plasma potassium were observed between early May and mid-May. In early May the peak occurred at S+6, with the trough at S+18. In mid-May the peak occurred at S+12, and the low point at S+6. In August the peak occurred at S+18 and the trough at S+12. The seasonal mean observed in mid-May was lower from the overall levels observed in any other season ($p = .01$).

Plasma potassium levels reported here for the white-throated sparrow, except for mid-May, are very close to those reported for man (Kramer and Tisdall, 1921) and the dog (Albritten, 1952). No values were found in the literature which were as low as those observed here in mid-May.

Brain derived data

Chloride space $(H_2O)_e$, intracellular water content $(H_2O)_c$, extracellular and intracellular sodium values, and the extra-intracellular sodium concentration ratios for mid-May and August are presented in Table XXVI. These data were analyzed only on the basis of fat-free fresh weight of the brain. The chloride space of the brain is defined as the extracellular water content, and is reported as grams of extracellular water per kilogram of fat-free fresh weight of the brain. The total water content of the brain minus the extracellular water gives an index of the intracellular water content. Intracellular water content was derived (see Methods and Materials) and converted to concentration in terms of mEq/kg intracellular water. From this value and the extracellular sodium concentration, the extra/intracellular sodium ratio was determined.

Significant diurnal variations of intracellular water, intracellular sodium concentration, extracellular sodium content, and extra/intracellular sodium concentration ratios were observed in mid-May. No rhythm of extracellular water was apparent. In August none of the derived data exhibited a diurnal rhythm when tested by an analysis of variance correlated with the photoperiod (Tables XXVIII through XXXVI).

The peak values in August were different when tested against the trough by Student's "t" test for intra- and extracellular sodium concentration and brain sodium ratio. The diurnal variations in August were much flatter than those found in mid-May and probably do not represent diurnal rhythms of these measurements.

In mid-May intracellular sodium concentration was lowest at S and highest from S+9 until midnight. The diurnal variation of intracellular sodium content was approximately 180^{o} out of phase from the extracellular sodium content. The extra/intracellular sodium concentration ratios were highest at S, and fell off sharply early in the day remaining low until S+18. These diurnal differences were verified by a Duncan's Multiple Range test. The grouping of this range test are given in Tables XXVIII through XXXI.

In August a single peak of intracellular sodium content occurred at S+6 and the trough occurred at S. The intracellular sodium concentration was highest at S+6 with lower levels at the other times of the day. The variation of extracellular sodium was out of phase with the diurnal change in intracellular sodium concentration: i.e. the lowest value occurred at S+6. The brain sodium ratio at S was higher ($p = .05$) from the lowest values at S+6 and S+18. However, none of these diurnal variations found in August were significant when tested with an analysis of variance against the photoperiod. The diurnal means of intracellular sodium concentration and brain sodium ratios are plotted against the photoperiod in Figures 5. and 6. No significant difference in the overall means of any of these measurements was found between mid-May and August.

The values for chloride space in the brain found in the white-throated sparrow were somewhat higher and the intracellular water content was lower than the values reported for rats (Woodbury, 1955; Timiras et al., 1954; Timiras et al., 1955; Woodward et al., 1967). The extra/intracellular sodium concentration ratio showed a great deal of variation ranging from 11.65 to 28.24 in the brain of the white-throated sparrow in mid-May.

In separate experiments Timiras (1954; 1955) reported brain extra/intracellular sodium concentration ratios of control rats as 7.1 and 17.5 respectively.

Since the brain potassium content is predominatly intracellular, the brain intracellular concentration of potassium could be calculated using the intracellular water values. These concentrations are presented in Table XXXVII. In mid-May the highest levels of intracellular potassium concentration occurred at S+9 and S+12; the lowest level, at S+6. The diurnal change was unimodal and was verified by an analysis of variance. The multiple range grouping is shown in Table XXXVIII. In August no significant diurnal change was apparent (Table XXXIX). Intracellular potassium concentration was plotted against the photoperiod in Figure 7.

The intracellular potassium concentration in mid-May was found to be lower ($p = .05$) than the level observed in August. The intracellular potassium concentration found in the white-throated sparrow (mid-May-182.1; August-189.1 mEq/liter intracellular water) is comparable to the value of 185.0 reported for the rat (Timiras et al., 1955; Woodbuy, 1955).

Discussion

Measurement of the diurnal levels of sodium, potassium, and chloride in the plasma and brain of the white-throated sparrow were made during three seasons of the year. The birds were known to be in different physiological states during the three seasons. In April the birds were photosensitive, but were not responding in a premigratory manner because the photoperiod was of insufficient length (12 hours, 27 minutes light). In mid-May the birds were physiologically prepared for migration and were responding to the photoperiod (13 hours, 42 minutes light). Preparedness to migrate was determined by visual inspection of heavy fat stores and observation of nocturnal locomotor activity. In August the photoperiod was still long (13 hours, 28 minutes light). The birds did not have heavy fat deposits, did not exhibit nocturnal locomotor activity but did have feather replacement of postnuptial molt. After the birds were killed the gonads were removed and weighed. Gonads from birds in mid-May were larger than those from birds in April or August.

In April and mid-May most of the electrolyte rhythms were the same shape. In August the rhythms were found to be different from the patterns observed earlier in the year. Differences were found both in phase and amplitude.

Seasonal variations in the diurnal rhythms of sodium and potassium cannot be attributed only to the photoperiod. There is a basic physiological difference between spring and late summer which is reflected in a shift of the diurnal rhythms of electrolytes in the white-throated sparrow.

Very few seasonal studies have taken into account possible diurnal rhythms and seasonal phase shifts of the criteria being examined. The potassium excretion rhythm in man was found to shift with the seasons (Ghata and Reinberg, 1954). More recently, studies of hormone levels in the white-throated sparrow have shown that seasonal phase shifting occurs in the diurnal rhythms of plasma corticosterone (Dusseau, 1969) and pituitary prolactin (Meier et al., 1969). Both a seasonal variation of the phase angle of the diurnal rhythm under examination and a possible amplitude change must be considered when interpreting data.

Seasonal comparisons of electrolyte content were made by using the mean of the diurnally collected samples. Use of the diurnal mean to make seasonal comparisons revealed fewer differences in electrolyte levels than would be evident if samples were taken only at one particular time of the day in each of the three months.

Diurnal rhythms of electrolytes

Brain content of sodium in April and mid-May vary significantly over the period of twenty-four hours. A rhythm of brain potassium content was evident in mid-May. In April a diurnal brain potassium variation seemed to be present but was not statistically verified. The diurnal peaks in these two months were similar in relation to the photoperiod. The peaks of brain sodium and potassium content occurred in the late afternoon. In August there was a damping out of the brain sodium rhythm, and an entirely different pattern of brain potassium content was present. In August a bimodal diurnal variation of brain potassium content occurred. The peaks occurred twelve hours apart, one at noon, and the other at midnight. Brain chloride content did not vary with the time of the day in mid-May or August, however, a small but significant, peak of brain chloride was observed at sunset in April.

The electrolyte patterns in April and mid-May were more comparable to one another than when either month was compared to August. In the spring months the physiological states were also similar. The August photoperiod was comparable in length to that of mid-May and both were longer than the April photoperiod.

The seasonal difference in electrolyte rhythms likely reflects a physiological difference rather than a direct response to the photoperiod; that is, electrolyte patterns were similar in birds which were physiologically similar, but exposed to different photoperiods. Birds exposed to similar photoperiods had different diurnal electrolyte patterns. A temporal phase shifting of factors responsible for physiological preparedness to migrate could cause the diurnal shift in electrolyte content of the brain.

Diurnal variations of plasma sodium occurred in both mid-May and August, and a diurnal variation of plasma potassium was evident in April, early May, mid-May, and August. The plasma sodium rhythm was damped and underwent a twelve-hour phase shift from mid-May to August. In mid-May plasma sodium was highest at sunrise, and in August the peak occurred during the night. A twelve-hour phase shifting was also evident in plasma potassium concentration. In April and mid-May the peak of plasma potassium occurred at noon, and in August the peak occurred at midnight and was lowest at noon. No rhythm of plasma chloride was apparent in either mid-May or August.

A diurnal rhythm of electrolyte excretion has been well documented in man (for review see Mills, 1966).

Most studies indicate decreased urine flow and decreased sodium, potassium, and chloride excretion at night. Excretion of these substances rises to a peak between 0900 and 1200, and then returns gradually to the night values. These excretion rhythms are not directly habit induced as they were present in nurses working on night shifts. Perhaps the measurements were taken before adequate time had elapsed to allow the electrolyte excretion rhythm to shift to the new time routine. Twenty-four hour excretion rhythms were persistent in subjects following a twelve-hour cycle of activity and sleep (Mills, 1951; Mills and Stanbury, 1952). They were present whether the subjects were sleeping or awake, and although they were magnified by the normal rhythm of recumbency, they were present whether the subject was recumbent or upright (Mills, 1953).

Evidence that the excretory rhythms are cued to external environmental factors come from studies of two types. After a jet flight from Amsterdam to New York, four days were required for the diurnal maximum of sodium, potassium, chloride, and water to shift from Amsterdam time to New York time (Gerritzen, 1962). Further, Ghata and Reinberg (1954) have stated that there are characteristic and reproducible seasonal differences in the diurnal rhythm of water and potassium excretion

in man. They found a shift of peak excretion from morning in autumn to afternoon in spring in subjects studied in Paris. Inasmuch as a transplated human kidney had the normal rhythm of sodium and water excretion (Gunn, 1960) a direct nervous control of the rhythm seems to be excluded. Thus, although the nervous system may not exert a direct influence over kidney function, it is probably involved in transmitting the photoperiodic information indirectly by controlling other factors which influence excretion rates.

Investigations of diurnal rhythms of human plasma electrolytes have not been very successful. The diurnal rhythms of human plasma electrolytes are usually irregular, notched, or biphasic, and always of very low amplitude and poorly developed (Mills and Stanbury, 1955; Wesson, 1964; Mills, 1966). More recent studies indicate that a well-defined diurnal rhythm of plasma chloride occurs in fish (Meier, personal communication).

A diurnal adjustment in the extra-intracellular distribution of these electrolytes may occur, thus preventing or at least lessening the effects of diurnal excretion rates. In the white-throated sparrow a diurnal rhythm of brain intracellular sodium concentration and brain sodium ratio exists in mid-May. As was the case

for brain sodium content, no rhythm is apparent in August.

In mid-May the highest brain sodium ratio occurred at sunrise. Brain sodium ratio fell steadily until sunset and remained low until midnight. Woodbury (1954) found that an increase in brain sodium ratio was correlated with decreased nervous sensitivity and that decreased brain sodium ratio was correlated with increased nervous sensitibity as measured by EST. A change in nervous sensitivity might result in an effect on locomotor activity. At all times of the year that diurnal locomotor activity of the sparrow was measured a peak of activity occurred at sunrise except in mid-May. The least amount of locomotor activity in mid-May occurred at sunrise. The morning peak of locomotor activity in mid-May was delayed until three hours after sunrise. No samples were taken at this time, however by noon the brain sodium ratio had fallen sharply and remained low until sunrise. During the time that the brain sodium ratio was falling, the locomotor activity was increasing and remained high until three hours before sunrise. A relatively insensitive nervous system at sunrise, with increased sensitivity during the day and first part of the night, is suggested.

Intracellular potassium concentration of the brain in mid-May was low from sunrise to noon, increased sharply during the afternoon, and remained high until midnight. Apparently an increase in intracellular sodium does not result in a concomitant decrease of intracellular potassium since the diurnal rhythm of intracellular sodium and potassium were parallel rather than reciprocal. The relationship of movements of potassium in the brain with nervous sensitivity is unclear. Woodbury (1957) found no relationship between extra-intracellular potassium movements and change in EST. There was no statistically significant rhythm of intracellular potassium in August.

The timing of locomotor activity in mid-May may be affected by changes in the sensitivity of the nervous system. The brain sodium ratio decreased during the time of day that locomotor activity increased. Brain sodium ratio was high at sunrise, when the least amount of locomotor activity occurred. The absence of a sunrise activity peak in mid-May might result from a highly insensitive nervous system due to a high brain sodium ratio at that time. The brain sodium ratio fell during the day and remained low during the night when nocturnal locomotor activity occurred. The proposed association between decreased brain sodium ratio and

increased locomotor activity is not evident at all times. Brain sodium ratio is low, and locomotor activity decreases during the afternoon in mid-May. Another system might inhibit locomotor activity during the afternoon in mid-May. There was no correlation of brain sodium ratio with locomotor activity in August. The system suggested for depressing locomotor activity in the afternoon in mid-May might undergo a seasonal phase shift, so that it inhibits locomotor activity during the night in August.

Seasonal electrolyte levels

The seasonal comparisons presented here utilize the diurnal mean for each season. The diurnal mean is a more accurate method of determining true quantitative seasonal differences than sampling at one time of the day in various seasons. It further emphasizes the importance of determining the diurnal rhythm of blood or brain factors before making seasonal comparisons. If seasonal comparisons are made this method has the advantage of blanking out phase shifts such as have been presented previously.

No seasonal difference in brain chloride content was found in April, mid-May, or August. Woolley and

Timiras (1964) reported an increase in brain chloride following sex hormone injections. The gonads in mid-May were enlarging but perhaps were not producing enough gonadal hormones to cause an increase in brain chloride. However, the oviducts of the females had begun to enlarge which indicates at least some estrogen secretion was occurring. No estimate of male sex hormone could be made. Because exogenous sex hormones have been shown to supress nocturnal locomotor activity in migratory birds (Wagner, 1961; Wagner and Thomas, 1957), and nocturnal locomotor activity occurs in castrates (Morton and Mewaldt, 1962), it seems possible that the titers of sex hormone in mid-May were not high enough to affect brain chloride.

Differences in plasma sodium and chloride between mid-May and August were evident. Plasma sodium concentration was higher and plasma chloride concentration was lower in mid-May than in August. It was expected that sodium and chloride would have similar seasonal and diurnal variations in both the plasma and the brain. Perhaps some other anion, such as bicarbonate, replaces chloride during mid-May. The increased metabolism during mid-May could provide enough bicarbonate to replace chloride.

The relationship between plasma sodium and chloride in birds is not clear. In mourning doves deprived of water for four days plasma sodium concentration rose slightly, but chloride concentration did not change. Further, drinking of 0.2M sodium chloride elevated the chloride levels of both plasma and urine but had no effect on sodium concentration in the plasma (Smyth and Bartholomew, 1966). In man sodium and chloride excretion appears to follow one another closely. Potassium and hydrogen ion excretion are thought to be related in a reciprocal manner by competing with each other for excretion by exchange with sodium (Mills and Stanbury, 1954). Voluntary hyperventilation in man can cause increased electrolyte excretion similar to that which occurs upon waking (Stanbury and Thompson, 1951).

The lowest potassium levels of both brain and plasma were observed in mid-May. Brain content was measured in April and August, and plasma concentrations were measured in April, early May, and August. The low level of brain potassium in mid-May represents lowered extracellular as well as intracellular concentrations. Woodbury and his co-workers found no correlation between nervous sensitivity as measured by EST and potassium content or concentration in the brain.

No quantitative seasonal differences were found for brain sodium content, intracellular sodium concentration, or brain sodium ratio. The most important seasonal difference of blood and brain electrolytes is not quantitative. Because the peaks and troughs may shift from season to season the diurnal rhythm can not be blanked out by taking samples only at one particular time in various seasons. Rather, the important seasonal differences are temporal. The diurnal timing of the phase angle and the amplitude of the rhythm must be considered when seasonal electrolyte changes are compared.

Possible endocrine control mechanisms

The fluctuations of sodium, potassium, and chloride in the plasma are resultants of several factors: diet, exchange with all the tissues, and excretion rates. The peak of plasma sodium in the morning in mid-May may be partly a result of increased water loss at night due to increased locomotor activity.

The diurnal levels of brain sodium, potassium, and chloride reflect changes in total water content of the brain and its movement to and from the extracellular space. Adrenocortical and/or thyroid hormones may be important in controlling the diurnal shift in ions from

one compartment to the other. Secretions from both of these glands have been implicated as an important part of the nocturnal locomotor activity phase of the migratory response, and they have also been shown to affect the sensitivity of the nervous system by affecting the extra-intracellular distribution of sodium.

It has been suggested that an effect of carbohydrate-active steroids is facilitation of polysynaptic nervous reactions (Henkin, 1968). Cortisone, cortisol, thyroxine, and triiodothyronine increase the sensitivity of the nervous system as measured by EST, by decreasing the brain sodium ratio (Woodbury, 1957). Terner and co-workers (1950) proposed that free glutamic and aspartic acid play an important role in the transport of sodium out of and potassium into brain cells. There is an increase in glutamine and asparagine and a decrease in glutamic and aspartic acid in the brain of cortisone and cortisol treated rats. Woodbury (1957) suggested that the mechanism of action of cortisol and cortisone on the brain was to decrease free glutamic and aspartic acid concentrations by converting them to glutamine and asparagine, respectively. If the acids were converted to amides they would not be available for active transport of sodium, and intracellular

brain sodium and nervous sensitivity would tend to rise. Treatment of rats with deoxycorticosterone acetate, a mineralocorticoid, results in increased brain sodium ratio and increased EST. Increases and decreases in EST were always associated with increased or decreased brain sodium ratio.

A diurnal rhythm of brain activity occurs in man. In normal man, there is a diurnal rhythm of total electroencephalograph (EEG) output with the greatest activity occurring during mid-morning (Frank et al., 1966). The daily activity cycle of the adrenal cortex has been correlated with a number of physiological changes. The peak of 17-hydroxycorticoids in the blood precedes abnormal EEG spikes of epileptic patients by about twelve hours (Engel, 1961). In mice the peak of blood corticosterone precedes the peak locomotor activity by approximately four hours, and in man both corticosterone and cortisol precede the peak of body activity by about five hours (Halberg et al., 1959; Halberg et al., 1959). These findings led Halberg to suggest that the adrenal cycle prepares the body for daily locomotor activity.

Seasonal changes in adrenal activity have been described. Histological evidence suggested increased adrenal activity in Gambel's white-crowned sparrow

during the winter, and decreased activity during the summer reproductive period (Lorenzen and Farner, 1964). Adrenal activity of migratory wagtails, measured histologically, was higher during the migratory periods than during the post-migratory periods (John, 1966). In the white-throated sparrow plasma corticosterone levels were highest in winter and decreased to a low level in August (Dusseau, 1969). Studies of nonmigratory birds have yielded different results. Increased cellular activity during the reproductive period has been reported for the starling (Burger, 1938), the Gambel quail (Raitt, 1968), and the European blackbird (Fromme-Bouman, 1962).

In companion studies to the ones presented here, corticosterone, the primary steroid secreted by the avian interrenal tissue (Nagra et al., 1960; deRoos, 1960, 1961), was determined quantitatively in the plasma of the same birds used in this study (Dusseau, 1969). Dusseau found a diurnal rhythm of corticosterone in mid-May with the peak at sunrise, and the low point about midnight. This diurnal variation of corticosterone levels is in high phase agreement with brain sodium ratio. In the white-throated sparrow corticosterone may act similarly to cortisone and cortisol in the rat by decreasing the brain sodium ratio,

thereby increasing nervous sensitivity. Because the brain sodium ratio remains low during nocturnal restlessness, the explanation for ionic regulation of nervous activity in rats (Woodbury, 1957) may also be applicable for nocturnal restlessness in the white-throated sparrow.

Woodbury found extra/intracellular sodium changes in rats six hours after injection of the test adrenocortical substance. The similarity of the phases of the diurnal rhythm of corticosterone (Dusseau, 1969) and brain sodium ratio indicate a similar temporal correlation in birds. Since no data was given by Woodbury concerning the possibility of a more rapid sodium response the time relationship between peak steroids in the blood and sodium movement is not discernable.

No rhythm of brain sodium ratio was found in August. Because corticosterone levels in August were found to have a diurnal rhythm with a similar phase relationship to that in mid-May the seasonal difference in brain sodium ratio cannot be attributed solely to corticosterone. Overall corticosterone levels in August were slightly lower than the levels found in mid-May. Although the times of the diurnal peaks during the two months were similar the time of the greatest disappear-

ance of corticosterone from the blood is different. In mid-May the time of greatest disappearance of corticosterone from the blood occurred between sunset and midnight. In August it occurred immediately after sunrise. The diurnal timing of the disappearance from the plasma may represent tissue uptake and utilization. Corticosterone and cortisol are known to be greatly concentrated in the tissues of the central nervous system of the cat (Touchstone et al., 1966). Perhaps corticosterone alone cannot change the brain sodium ratio. The sodium movement may be due to a synergism of corticosterone with another factor. A seasonal phase shift in the second factor might be responsible for the absence of the diurnal rhythm of the brain sodium ratio in August.

A number of the physiological changes associated with the preparedness to migrate have been shown to be both photoperiod dependent (Rowan, 1926, 1932, 1946; Farner, 1950, 1955; King and Farner, 1963), and hormone dependent (Woolfson, 1945; Meier and Farner, 1964; Meier et al., 1965). It has also been demonstrated that premigratory fattening and nocturnal restlessness are dependent upon the time of day that exogenous prolactin is injected (Meier and Davis, 1966; Meier, 1969). Pituitary prolactin in white-throated sparrows

having heavy vernal fat stores was found to be released from the pituitary after the middle of the photoperiod. Injection of exogenous prolactin at the middle of the photoperiod caused fattening of photorefractory birds. Release of endogenous prolactin in photorefractory birds in August occurred twelve hours later than release of pituitary prolactin in photosensitive birds in mid-May (Meier et al., 1969). Prolactin must be available at the correct time of day in relation to the phase of another substance which synergizes with prolactin to produce fattening.

The possibility that a functional integration between prolactin and corticosterone might exist was discussed by Dusseau (1969). Prednisone, a synthetic glucocorticoid very similar to cortisone chemically and functionally, augmented nocturnal locomotor activity induced out of season by prolactin in the white-crowned sparrow. Prednisone was ineffective alone (Meier et al., 1965). Further, induced nocturnal locomotor activity of photosensitive white-crowned sparrows was reduced or completely eliminated with metapirone, a synthetic compound that selectively inhibits 11-hydroxylation during steroid synthesis and decreases plasma corticosterone levels in the plasma of the pheasant (Nagra et al., 1963).

Prolactin induction of nocturnal locomotor activity was also time dependent. Prolactin treatment at the middle or end of the day in April induced locomotor activity, whereas prolactin given shortly after sunrise was completely ineffective (Meier, 1969). A specific temporal relationship between corticosterone and prolactin is apparently necessary for induction of locomotor activity. The effect of the steroid on the nervous system may be dependent on prolactin, or prolactin might phase the action of corticosterone. Peak levels of pituitary prolactin in mid-May were found at noon and fell during the next nine hours (Meier et al., 1969). It was suggested that endogenous prolactin was released into the blood during the afternoon. Thus in mid-May prolactin release follows the peak of plasma corticoseterone by six hours. Late afternoon was the time of greatest disappearance of corticosterone from the blood. Prolactin might time the disappearance from the blood by causing the nervous system to accumulate corticosterone at that time. In August the corticosterone rhythm was very similar to that found in mid-May, but the release of endogenous prolactin occurred between midnight and sunrise. The time of the greatest disappearance of corticosterone from the blood occurred just after sunrise. The phase

relationship between prolactin and corticosterone in mid-May may be necessary for the expression of a diurnal rhythm of brain sodium.

Effects of these hormones on brain sodium ratio cannot be the only system which affects the timing of locomotor activity. Brain sodium ratio and locomotor activity are both low during the late afternoon in mid-May; further, in August no correlation of brain sodium ratio with locomotor activity exists. In recent studies melatonin was found to decrease the amount of locomotor activity in the house sparrow held on constant light (Meier, personal communication). Melatonin release might inhibit locomotor activity in the late afternoon in mid-May. Timing of the release of melatonin might shift between mid-May and August and result in inhibiting locomotor activity during the night in August.

A diurnal rhythm of brain sodium ratio in mid-May and the absence of a rhythm in August, associated with a seasonal phase shifting of the rhythm of prolactin in the white-throated sparrow, would suggest that experimental studies of the effect of prolactin on electrolyte distribution would be of interest. Experimental evidence of the possible effect of prolactin on electrolytes in birds is lacking. Prolactin injections are necessary to prevent negative sodium

balance in hypophysectomized Fundulus, a euryhaline fish, in fresh water (Maetz et al., 1967). Prolactin decreased sodium loss by the gills (Potts and Evans, 1966) and by the kidney (Stanley and Fleming, 1966) in different species of Fundulus. No work has been done on the possible effects of prolactin on the extra-intracellular distribution of sodium. A diurnal rhythm of the brain sodium ratio which may be associated with locomotor activity is present in mid-May in the white-throated sparrow. Further, prolactin given at the proper time can induce nocturnal locomotor activity in birds. The diurnal rhythm of extra-intracellular sodium distribution of birds exhibiting nocturnal locomotor activity induced by prolactin would be of great interest.

Table I. Seasonal Variations in Body Weight, Lipid Index, Gonadal Weights, and Molt in White-throated Sparrows Maintained in Outdoor Aviaries

	Body Weight (grams)	Lipid Index (% Dry Weight)	Paired Testes (mg)	Ovary (mg)	Oviduct (mg)	Molt	NLA*
April 5	24.8	17.5	2.6	12.5	4.1	Pre-nuptial	-
May 5	27.5	42.9				None	-
May 15	30.5	53.4	46.0	23.8	10.7	None	+
August 7	25.7	15.7	3.5	13.4	4.3	Post-nuptial	-

*Nocturnal Locomotor Activity

Table II. Diurnal and Seasonal Levels of Brain Electrolyte Content in April, Mid-May, and August in the White-throated Sparrow

April

Time		Na	K	Cl	H_2O[4]
S[1]	(5)[2]	52.79±0.86[3]	102.77±2.58	33.12±1.41	803.2±1.7
S+6	(6)	54.62±0.77	101.19±1.82	34.56±0.13	803.5±1.8
S+12	(6)	56.18±1.81	106.36±3.56	37.24±1.49	797.9±4.7
S+18	(6)	51.95±0.76	105.84±1.69	34.25±0.79	799.5±1.2
Overall		53.93±0.44	104.10±1.26	34.90±0.60	801.1±1.3

Mid-May

S	(6)	51.12±0.37	93.50±0.90	33.78±1.21	809.3±1.8
S+6	(5)	51.95±0.69	91.73±2.44	33.65±0.93	814.8±3.2
S+9	(5)	56.15±0.87**	98.34±1.23	35.83±0.81	801.1±1.3
S+12	(5)	53.93±1.08	98.66±1.39*	35.76±0.87	800.0±1.0
S+15	(6)	52.06±0.97	96.60±1.45	34.79±1.81	807.4±0.9
S+18	(6)	51.51±0.99	98.37±0.72	34.45±0.64	806.2±1.0
Overall		52.67±0.44	96.19±0.71	34.68±0.37	806.2±1.0

August

S	(7)	51.36±1.12	97.01±0.85	37.63±0.60	805.2±0.8
S+6	(5)	51.46±0.78	101.72±1.42*	36.53±1.07	806.3±1.7
S+12	(6)	51.72±0.54	97.56±0.46	36.45±0.49	808.4±0.8
S+18	(5)	50.68±1.94	103.67±2.30*	35.62±1.59	804.1±2.3
Overall		51.33±0.55	99.62±0.84	36.64±0.46	806.0±0.7

1. S-Sunrise.
2. Number of individuals in each group.
3. Means in mEq/kg fat-free fresh brain weight ± S.E.
4. Means in g/kg fat-free fresh brain weight ± S.E.

Peak value is significantly different from the lowest value at least at the 95% (*) or the 99% (**) confidence interval by Student's "t" test.

Table III. Diurnal and Seasonal Levels of Brain Electrolyte Content in April, Mid-May, and August in the White-throated Sparrow

April

Time		Na	K	Cl	H_2O
S	(5)[1]	50.37±0.85[2]	96.90±2.39	31.39±1.32	757.7±1.9[3]
S+6	(6)	51.55±0.73	95.84±1.50	32.61±0.37	758.1±1.8
S+12	(6)	52.92±1.62	100.20±3.10	34.26±1.11	746.0±8.6
S+18	(6)	49.01±0.67	99.84±1.53	32.32±0.73	754.2±1.8
Overall		50.99±0.59	98.25±1.11	32.70±0.48	753.8±2.4

Mid-May

S	(6)	48.24±0.27	88.38±0.80	31.92±0.74	765.0±1.7
S+6	(5)	49.15±0.67	86.93±2.71	31.89±0.87	771.6±3.0
S+9	(5)	53.66±0.83	93.97±1.16	34.24±0.62	765.8±1.4
S+12	(5)	51.25±1.03	93.78±0.85	33.99±1.16	760.2±1.3
S+15	(6)	49.45±0.93	92.91±0.62	33.05±0.88	767.0±1.3
S+18	(6)	48.99±0.93	93.54±0.67	32.77±0.77	764.8±0.9
Overall		50.05±0.44	91.59±0.68	32.94±0.36	765.7±0.8

August

S	(7)	48.83±1.07	91.94±0.81	35.68±0.58	763.3±0.7
S+6	(5)	48.76±0.76	96.38±1.35	34.62±1.06	763.9±2.1
S+12	(6)	48.92±0.53	92.27±0.42	34.48±0.47	764.7±1.4
S+18	(5)	48.14±2.04	98.43±2.48	33.83±1.62	762.5±1.4
Overall		48.69±0.55	94.40±0.84	34.73±0.46	763.5±0.6

1. Number of individuals in each group.
2. Means in mEq/kg fresh brain weight ± S.E.
3. Means in g/kg fresh brain weight ± S.E.

Table IV. Analysis of Variance and Duncan's New Multiple Range Test of Brain Sodium Content at 4 Times of the Day in April

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	93.2130	31.0710	5.5085
Error	18	101.5293	5.6405	
Total	21	194.7423		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 1.0126$

Value of p	2	3	4
SSR	2.97	3.12	3.21
R_p=LSR	3.0074	3.1593	3.2504

S+18	S	S+6	S+12
51.94	52.79	54.62	56.18

Table V. Analysis of Variance of Brain Potassium Content at 4 Times of the Day in April

	Analysis of Variance			
Source	df	SS	MS	F
Hours after sunrise	3	145.3798	48.4599	1.3752*
Error	18	634.2494	35.2360	
Total	21	779.6292		

*Not significant

Table VI. Analysis of Variance and Duncan's New Multiple Range Test of Brain Chloride Content at 4 Times of the Day in April

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	63.5809	21.1936	3.3377
Error	18	114.2957	6.3497	
Total	21	177.8767		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 1.0744$

Value of p	2	3	4
SSR	2.97	3.12	3.21
R_p=LSR	3.1909	3.3521	3.4488

S	S+18	S+6	S+12
33.28	34.26	34.56	37.98

Table VII. Analysis of Variance of Brain Water Content at 4 Times of the Day in April

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	108228.9912	36076.3304	1.2891*
Error	18	503740.0033	27985.5557	
Total	21	611968.9945		

*Not significant

Table VIII. Analysis of Variance and Duncan's New Multiple Range Test of Brain Sodium Content at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	95.7560	19.1512	4.7444
Error	27	108.9857	4.0365	
Total	32	204.7418		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 0.8566$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.205	3.265
R_p=LSR	2.4841	2.6126	2.6851	2.7454	2.7967

S	S+18	S+6	S+15	S+12	S+9
51.12	51.51	51.95	52.06	53.93	56.15

Table IX. Analysis of Variance and Duncan's New Multiple Range Test of Brain Potassium Content at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	225.8078	45.1615	3.9170
Error	27	311.2946	11.5294	
Total	32	537.1025		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 1.4478$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.206	3.265
R_p=LSR	4.1986	4.4157	4.5388	4.6401	4.7270

S+6	S	S+15	S+18	S+9	S+12
91.73	93.50	96.60	98.36	98.34	98.66

Table X. Analysis of Variance of Brain Chloride Content at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	23.0643	4.6128	1.0190*
Error	27	122.2143	4.5264	
Total	32	145.2786		

*Not significant

Table XXI. Diurnal Levels of Plasma Potassium Concentrations in April, Early May, Mid-May, and August in the White-throated Sparrow

Time	April		Early May		Mid-May		August	
S	(5)[1]	2.98±0.30[2]	(6)	4.68±0.26	(6)	1.72±0.21	(12)	4.56±0.28
S+6	(6)	3.21±0.41	(6)	5.73±0.28*	(6)	1.67±0.04	(11)	4.01±0.32
S+9					(6)	2.73±0.31		
S+12	(5)	4.42±0.26*	(6)	3.85±0.41	(6)	2.83±0.33*	(10)	3.50±0.48
S+15			(5)	3.70±0.45	(6)	2.42±0.13		
S+18	(6)	4.06±0.30	(6)	3.55±0.22	(6)	2.23±0.29	(12)	4.63±0.26*
Overall		3.67±0.20		4.32±0.21		2.28±0.12+		4.21±0.17

1. Number of individuals in each group.

2. Means in mEq/liter plasma ± S.E.

* Peak value is statistically different from the lowest value at least at the 99% confidence interval by Student's "t" test.

\+ Overall mean in mid-May is significantly different from the means of the other three months at least at the 99% confidence interval by Student's "t" test.

Table XI. Analysis of Variance and Duncan's New Multiple Range Test of Brain Water Content at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	773.2564	154.6512	9.5968
Error	27	435.0986	16.1147	
Total	32	1208.3551		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 1.7116$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.205	3.265
R_p=LSR	4.9636	5.2203	5.3658	5.4856	5.5883

S+12	S+9	S+18	S+15	S	S+6
800.0	801.2	804.1	807.8	809.3	814.3

Table XII. Analysis of Variance of Brain Sodium Content at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	3.0854	1.0285	1.0285*
Error	19	148.5930	7.8207	
Total	22	151.6784		

*Not significant

Table XIII. Analysis of Variance and Duncan's New Multiple Range Test of Brain Potassium Content at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	177.4660	59.1553	6.1571
Error	19	182.5452	9.6076	
Total	22	360.0112		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 1.2982$

Value of p	2	3	4
SSR	2.96	3.11	3.19
R_p=LSR	3.8426	4.0374	4.1412

S	S+12	S+6	S+18
97.01	97.56	101.72	103.62

Table XIV. Analysis of Variance of Brain Chloride Content at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	12.3471	4.1157	0.8137*
Error	19	96.1007	5.0579	
Total	22	108.4478		

*Not significant

Table XV. Analysis of Variance of Brain Water Content at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	59.4901	19.8300	1.6602*
Error	19	226.9386	11.9441	
Total	22	286.4287		

*Not significant

Table XVI. Diurnal Levels of Plasma Sodium and Chloride Concentrations in Mid-May and August in the White-throated Sparrow

Time	Mid-May			August		
		Na	Cl		Na	Cl
S	(6)[1]	184.8±1.3*[2]	121.7±3.0	(6)	167.0±0.7	129.3±2.3
S+6	(6)	182.2±1.4	119.9±1.9	(5)	168.4±3.5	129.4±3.1
S+9	(6)	177.7±2.3	120.5±1.6			
S+12	(6)	171.8±1.4	121.0±1.5	(4)	172.7±1.6	125.9±1.3
S+15	(6)	170.7±1.0	117.1±1.5			
S+18	(6)	167.5±0.5	119.9±1.9	(6)	173.6±1.9*	128.0±4.9
Overall		175.8±1.2+	119.9±0.8+		170.3±1.1	127.5±1.0

1. Number of individuals in each group

2. Means in mEq/liter plasma ± S.E.

* Peak value is significantly different from the lowest value at least at the 99% confidence interval by Student's "t" test

\+ Overall Mid-May mean is significantly different from the overall August mean at least at the 99% confidence interval by Student's "t" test

Table XVII. Analysis of Variance and Duncan's New Multiple Range Test of Plasma Levels of Sodium at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	1417.5555	283.9111	21.6910
Error	30	392.6666	13.0888	
Total	35	1812.2222		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 1.4769$

Value of p	2	3	4	5	6
SSR	2.89	3.04	3.12	3.20	3.25
R_p=LSR	4.2682	4.4897	4.6079	4.7260	4.7999

S+18	S+15	S+12	S+9	S+6	S
167.5	170.7	171.8	177.7	182.2	184.8

Table XVIII. Analysis of Variance of Plasma Levels of Chloride at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	66.9555	13.3911	0.5504*
Error	30	729.7900	24.3263	
Total	35	796.7455		

*Not significant

Table XIX. Analysis of Variance and Duncan's New Multiple Range Test of Plasma Levels of Sodium at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	175.3833	58.4611	2.6912
Error	17	369.2833	21.7225	
Total	20	544.6666		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 2.0341$

Value of p	2	3	4
SSR	2.98	3.13	3.22
R_p=LSR	6.0616	6.3667	6.5498

S	S+6	S+12	S+18
167.0	168.4	172.8	173.7

Table XX. Analysis of Variance of Plasma Levels of Chloride at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	37.5811	12.5270	0.4692*
Error	17	453.8616	26.6977	
Total	20	491.4428		

*Not significant

Table XXII. Analysis of Variance and Duncan's New Multiple Range Test of Plasma Levels of Potassium at 4 Times of the Day in April

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	7.3700	2.4566	4.42123
Error	18	10.4976	0.5832	
Total	21	17.8677		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 0.3255$

Value of p	2	3	4
SSR	2.97	3.12	3.21
R_p=LSR	.9667	1.0155	1.0448

S	S+6	S+18	S+12
2.98	3.22	4.07	4.42

Table XXIII. Analysis of Variance and Duncan's New Multiple Range Test of Plasma Levels of Potassium at 5 Times of the Day in Early May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	4	19.5814	4.8953	7.74682
Error	24	15.7316	0.6554	
Total	28	35.3131		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 0.3361$

Value of p	2	3	4	5
SSR	2.92	3.07	3.15	3.22
R_p=LSR	0.9814	1.0318	1.0587	1.0822

S+18	S+15	S+12	S	S+6
3.55	3.70	3.85	4.68	5.73

Table XXIV. Analysis of Variance and Duncan's New Multiple Range Test of Plasma Levels of Potassium at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	6.9955	1.3991	3.4555
Error	30	12.1466	0.4048	
Total	35	19.1422		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = .2596$

Value of p	2	3	4	5	6
SSR	2.89	3.04	3.12	3.20	3.25
R_p=LSR	0.7502	0.7891	0.8099	0.8307	0.8437

S	S+6	S+18	S+15	S+9	S+12
1.72	1.72	2.25	2.42	2.73	2.83

Table XXV. Analysis of Variance and Duncan's New Multiple Range Test of Plasma Levels of Potassium at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	8.9505	2.9835	2.4725
Error	41	49.4739	1.2067	
Total	44	58.4244		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 0.3274$

Value of p	2	3	4
SSR	2.86	3.01	3.10
R_p=LSR	.9363	.9854	1.0149

S+12	S+6	S	S+18
3.51	4.01	4.56	4.63

Table XXVI. Diurnal Variation of Extracellular Water Content (Chloride Space), Intracellular Water, Intra- and Extracellular Sodium Content, Intracellular Sodium Concentration, and Extra- Intracellular Sodium Concentration Ratios of White-throated Sparrows Sacrificed in Mid-May and August

Time and Season	Water		Intracellular Na		Extracellular Na	Extra/Intracellular Na Ratio
	Cl Space $(H_2O)_e$	Intracellular Water $(H_2O)_c$	Na Content $(Na)_c$	Na Concentration $(Na)_c$	Na Content $(Na)_e$	$(Na)_e/(Na)_c$
Mid-May						
S (6)[1]	267.6±3.8[2]	541.6±5.4	3.62±0.50	6.66±0.86	47.50±0.52	28.42±2.53**
S+6 (5)	267.1±3.4	547.7±5.7**	5.08±0.48	9.30±0.91	46.87±0.67	19.74±2.26
S+9 (5)	285.3±2.3	515.9±2.7	7.47±1.28*	14.53±2.52*	46.88±0.48	14.47±4.16
S+12 (5)	281.3±8.0	518.8±7.6	7.47±0.48*	14.36±0.73	46.46±1.54	11.65±0.75
S+15 (6)	285.3±6.7	522.1±6.9	5.33±0.70	10.04±1.23	46.73±1.06	17.92±2.63
S+18 (6)	276.1±7.2	528.0±7.6	7.12±0.89	13.43±1.56	44.40±1.16	13.07±1.91
Overall	277.1±2.5	529.2±3.1	5.96±0.39	11.26±0.74	46.72±0.43	17.77±2.49
August						
S (6)	283.4±3.6	521.4±3.1	4.88±0.66	9.35±1.09	45.42±0.53	18.61±2.57*
S+6 (5)	271.0±4.9	535.3±3.4	7.68±0.99*	14.36±1.87*	43.78±0.66	12.09±1.66
S+12 (4)	278.7±6.8	529.8±5.7	5.17±0.23	9.76±0.40	46.19±0.52*	17.08±0.64
S+18 (5)	268.2±9.9	535.9±11.1	6.30±1.03	11.79±2.01	44.39±1.54	15.59±2.36
Overall	275.5±3.0	530.2±3.3	5.99±0.45	11.29±0.84	44.90±1.54	15.92±1.13

1. Number of individuals in each group.
2. Values represent means calculated from each individual based on fat-free fresh weight of the brain ± S.E. Water content is expressed as g/kg tissue; sodium content is expressed as mEq/kg tissue, and sodium concentration is expressed as mEq/liter of cellular water $(H_2O)_c$.

Peak value is significantly different from the lowest value at least at the 95%(*), or the 99%(**) confidence interval by Student's "t" test.

Table XXVII. Analysis of Variance of Brain Extracellular Water Content at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	1859.1800	371.8360	1.9939*
Error	27	5034.9696	186.4803	
Total	32	6894.1496		

*Not significant

Table XXVIII. Analysis of Variance and Duncan's New Multiple Range Test of Brain Cellular Water Content at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	4363.9621	872.7924	3.8794
Error	27	6074.4400	224.9793	
Total	32	10438.4024		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 6.3957$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.205	3.265
R_p=LSR	18.5475	19.5068	20.0505	20.4982	20.8819

S+9	S+12	S+15	S+18	S	S+6
515.9	518.8	522.1	528.0	541.6	547.7

Table XXIX. Analysis of Variance and Duncan's New Multiple Range Test of Brain Sodium Extracellular Content at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	55.6684	11.1337	2.1024
Error	27	142.9832	5.2957	
Total	32	198.6516		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 0.9267$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.205	3.265
R_p=LSR	2.6874	2.8264	2.9052	2.9700	3.0256

S+18	S+12	S+15	S+6	S	S+9
44.40	46.46	46.73	46.87	47.50	48.68

Table XXX. Analysis of Variance and Duncan's New Multiple Range Test of Sodium Intracellular Concentration at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	284.5901	56.9180	5.1996
Error	27	295.5585	10.9465	
Total	32	580.1460		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 1.4107$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.205	3.265
R_p=LSR	4.0910	4.3026	4.4225	4.5212	4.6059

S	S+6	S+15	S+18	S+12	S+9
6.66	9.30	10.04	13.43	14.36	14.53

Table XXXI. Analysis of Variance and Duncan's New Multiple Range Test of $[Na]_e/[Na]_c$ in the Brain at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	1051.8369	210.3674	5.8557
Error	27	969.9785	35.9251	
Total	32	2021.8155		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 2.5557$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.205	3.265
R_p=LSR	7.4115	7.7948	8.0121	8.1910	8.3443

S+12	S+18	S+9	S+15	S+6	S
11.65	13.07	14.47	17.92	19.74	28.24

Table XXXII. Analysis of Variance of Brain Extracellular Water Content at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	782.8937	260.9646	1.5635*
Error	16	2670.6358	166.9141	
Total	19	3453.5295		

*Not significant

Table XXXIII. Analysis of Variance of Brain Intracellular Water Content at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	760.3921	253.4640	1.1897*
Error	16	3408.5133	2130.0320	
Total	19	4168.9055		

*Not significant

Table XXXIV. Analysis of Variance of Brain Sodium Extracellular Content at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	15.8845	5.2948	1.2569*
Error	16	67.3995	4.2124	
Total	19	83.2841		

*Not significant

Table XXXV. Analysis of Variance of Brain Sodium Intracellular Concentration at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	80.2996	26.7665	2.2762*
Error	16	188.1435	11.7589	
Total	19	268.4431		

*Not significant

Table XXXVI. Analysis of Variance of $[Na]_e/[Na]_c$ in the Brain at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	122.6402	40.8800	1.7907*
Error	16	365.2625	22.8289	
Total	19	487.9028		

*Not significant.

Table XXXVII. Diurnal Variation of Intracellular Potassium Concentration of the Brain of White-throated Sparrows Sacrificed in Mid-May and August

Time	Mid-May		August	
S	(6)[1]	172.8±2.7[2]	(6)	187.2±1.2
S+6	(5)	167.7±6.4	(5)	190.0±1.9
S+9	(5)	190.7±3.2**		
S+12	(5)	190.4±3.5	(4)	184.7±2.1
S+15	(6)	185.3±4.6		
S+18	(6)	186.5±3.4	(5)	194.1±8.0
Overall		182.1±2.1+		189.1±2.1

1. Number of individuals in each group.

2. Means in mEq/liter of intracellular water based on fat-free fresh weight of the brain ± S.E.

** Peak value is significantly different from the lowest value at least at the 99% confidence interval.

+ May overall mean is significantly different from the August overall mean at least at the 95% confidence interval.

Table XXXVIII. Analysis of Variance and Duncan's New Multiple Range Test of Brain Potassium Intracellular Concentration at 6 Times of the Day in Mid-May

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	5	2446.3634	489.2727	5.2020
Error	27	2539.4590	94.0540	
Total	32	4985.8224		

Duncan's Multiple Range - 95% Confidence Interval

$S_x = 4.1352$

Value of p	2	3	4	5	6
SSR	2.90	3.05	3.135	3.205	3.265
R_p=LSR	11.9920	12.6123	12.9638	13.2533	13.5014

S+6	S	S+15	S+18	S+12	S+9
167.7	172.8	185.3	186.5	190.4	190.7

Table XXXIX. Analysis of Variance of Brain Potassium Intracellular Concentration at 4 Times of the Day in August

Analysis of Variance

Source	df	SS	MS	F
Hours after sunrise	3	231.8662	77.2887	0.8398*
Error	16	1472.4993	92.0312	
Total	19	1704.3655		

*Not significant

Figure 1.

The diurnal activity pattern of caged white-throated sparrows in April, early May, mid-May, and August. The activity index is the mean number of 2 minute intervals per hour with 3 or more hops. Locomotor activity in April is the mean of 4 birds for 5 nights; in early May 4 birds for 7 nights; in mid-May 3 birds for 5 nights; and in August 6 birds for 6 nights.

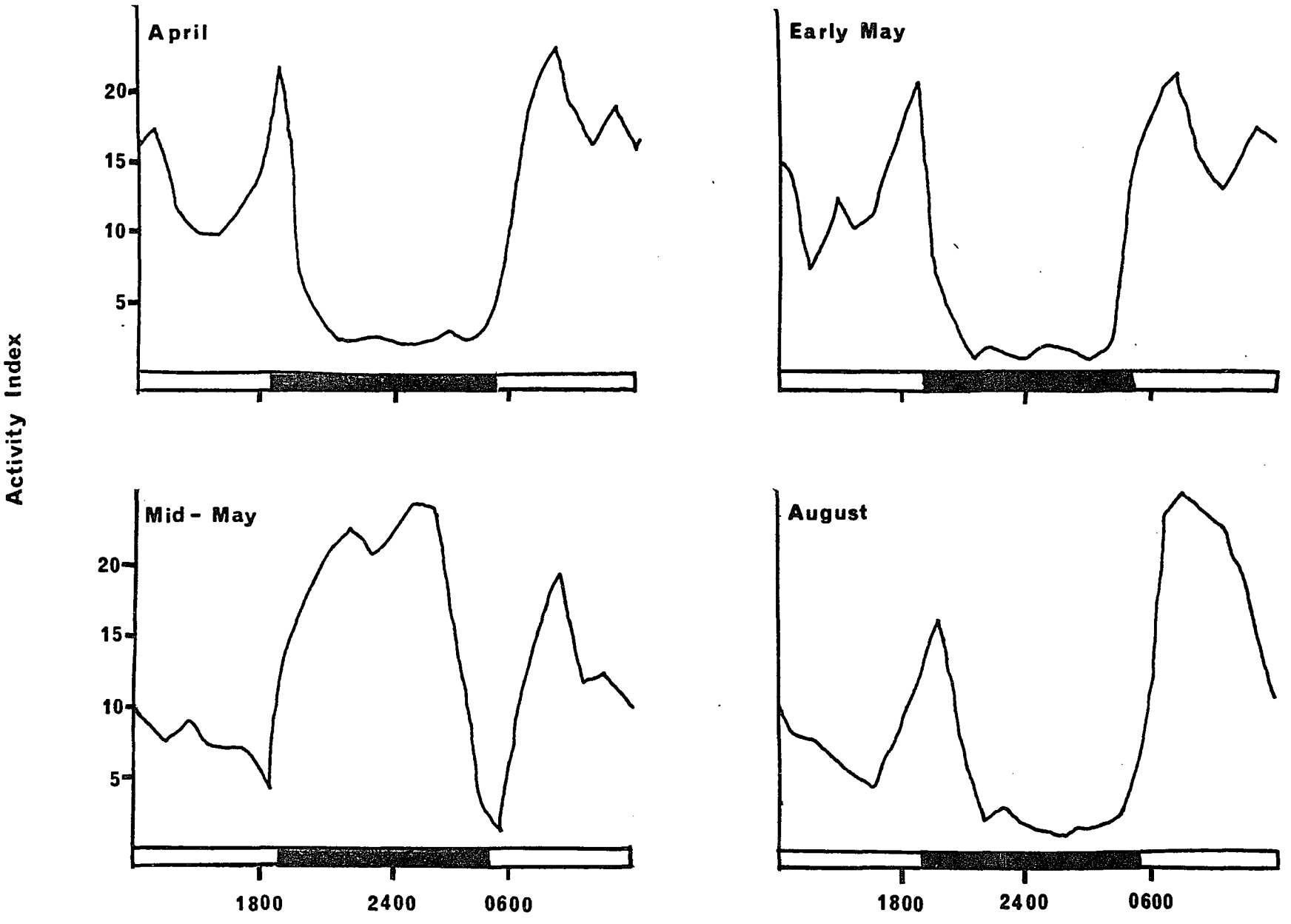

April
Early May
Mid - May
August
Activity Index
20
15
10
5
1800
2400
0600

Figure 2.

Diurnal rhythm of brain sodium content of the white-throated sparrow in April, mid-May, and August. The values are expressed as the mean in mEq/kg fat-free fresh brain weight $\pm$ S.E. The respective photoperiods are indicated on the abscissa.

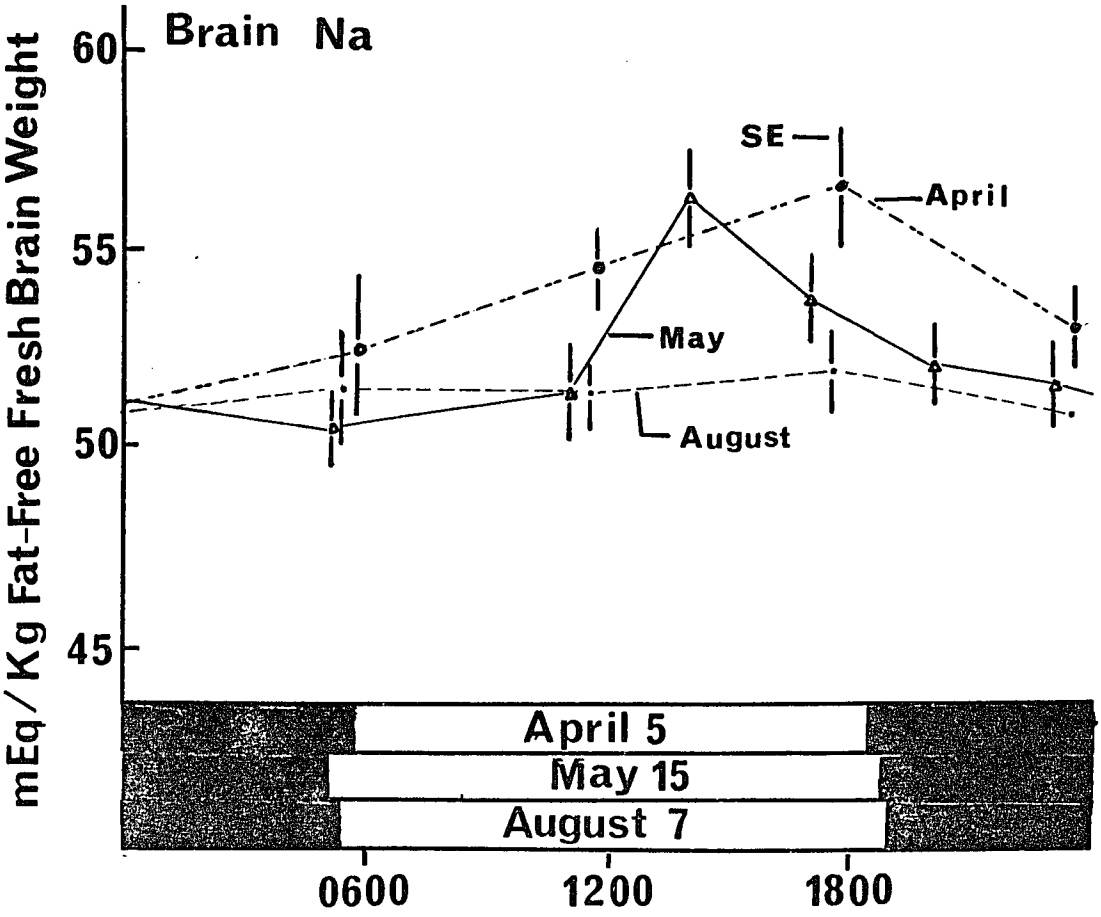

Brain Na
mEq / Kg Fat-Free Fresh Brain Weight
60
55
50
45
SE
April
May
August
April 5
May 15
August 7
0600
1200
1800

Figure 3.

Diurnal Rhythm of brain potassium content of the white-throated sparrow in April, mid-May, and August. The values are expressed as the mean in mEq/kg fat-free fresh brain weight $\pm$ S.E. The respective photoperiods are indicated on the abscissa.

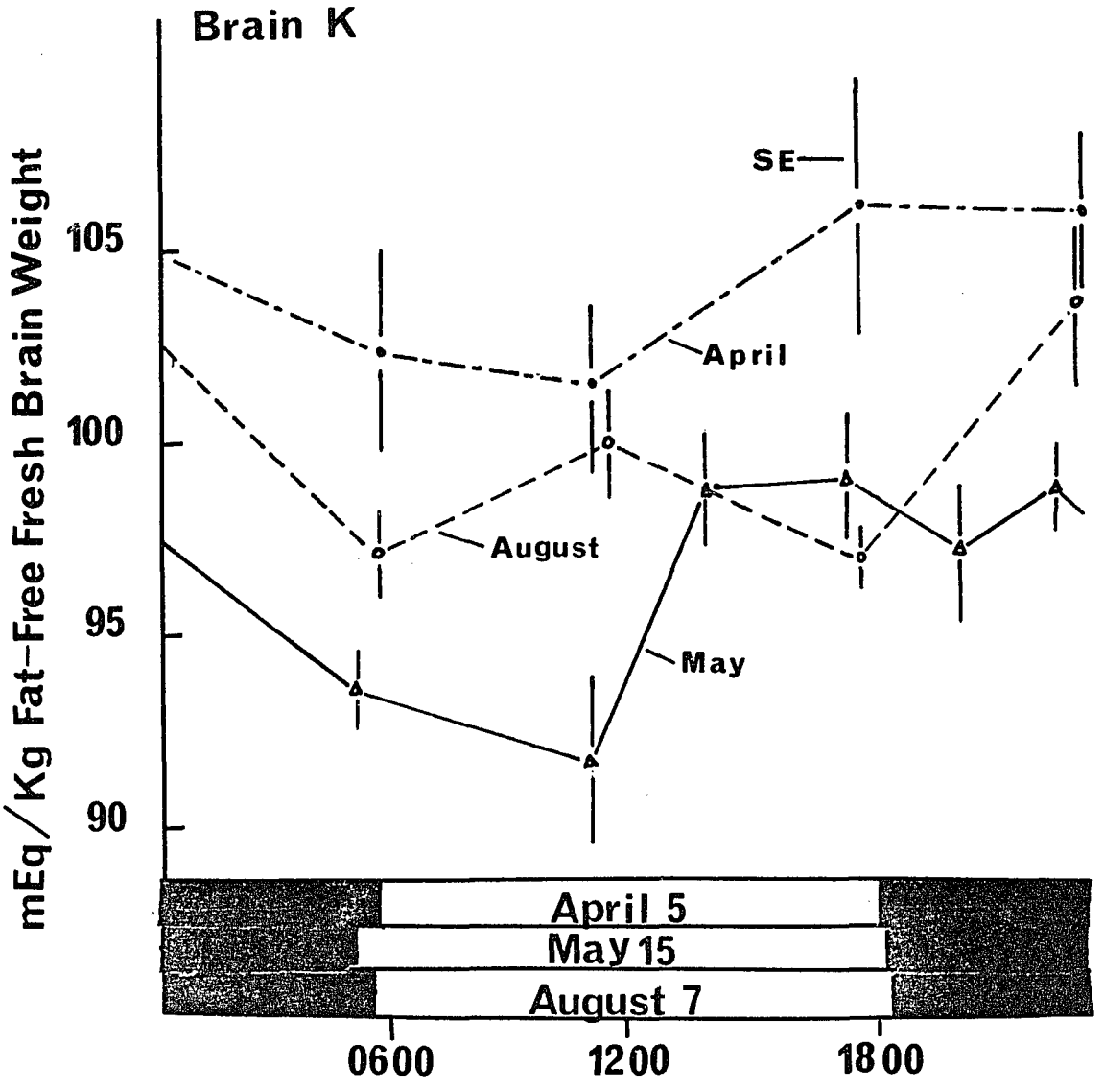

Brain K
mEq/Kg Fat-Free Fresh Brain Weight
105
100
95
90
SE
April
August
May
April 5
May 15
August 7
0600
1200
1800

Figure 4.

Diurnal rhythm of plasma sodium in the white-throated sparrow in mid-May and August. Values represent concentration as percentages about the total daily means. The respective photoperiods are indicated along the abscissa.

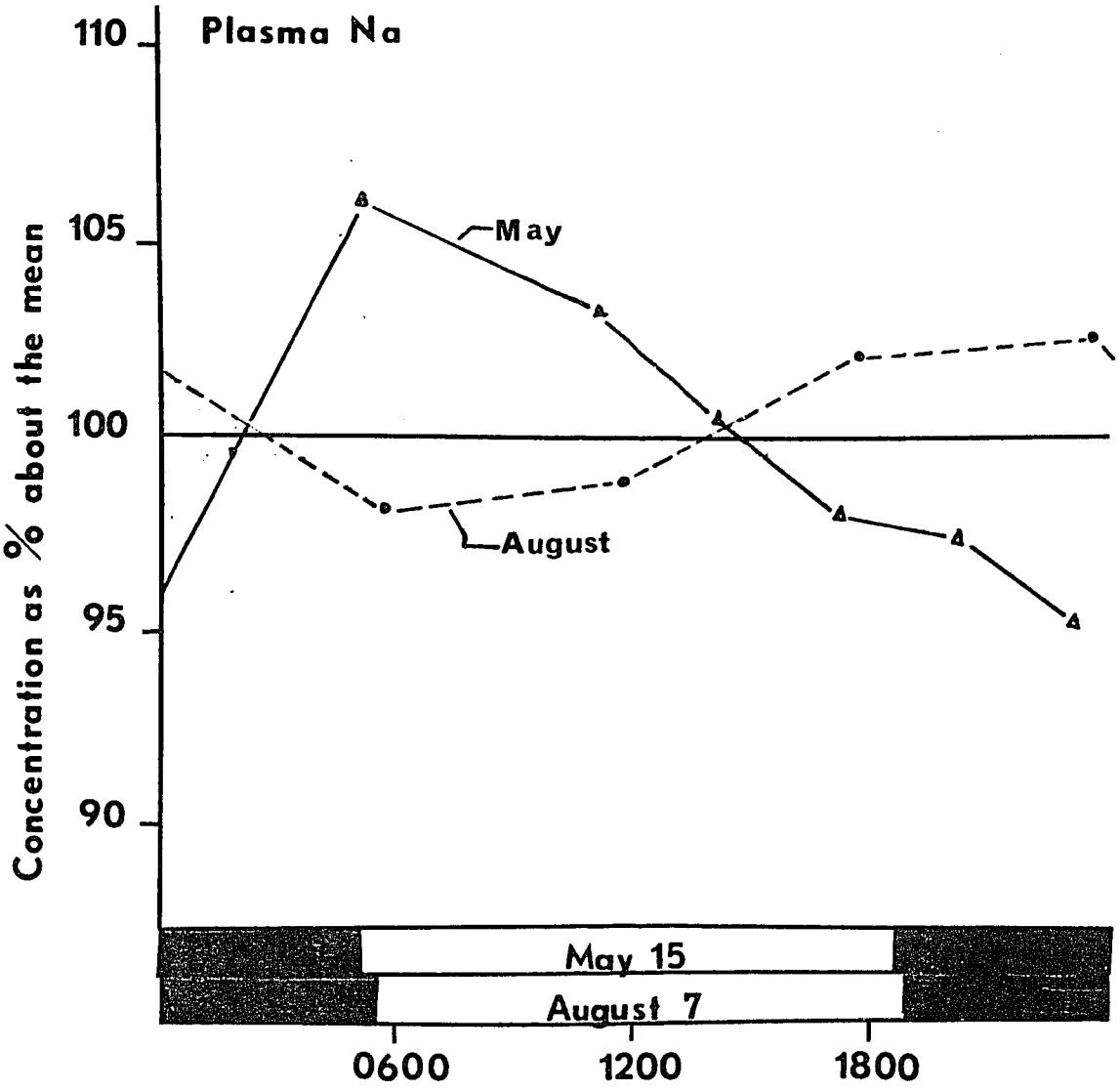

Plasma Na
110
105
100
95
90
Concentration as % about the mean
May
August
May 15
August 7
0600
1200
1800

Figure 5.

The diurnal rhythm of intracellular sodium concentration in the brain of white-throated sparrows in mid-May and August. Values represent concentration as mEq/liter intracellular water + S.E. The respective photoperiods are indicated on the abscissa.

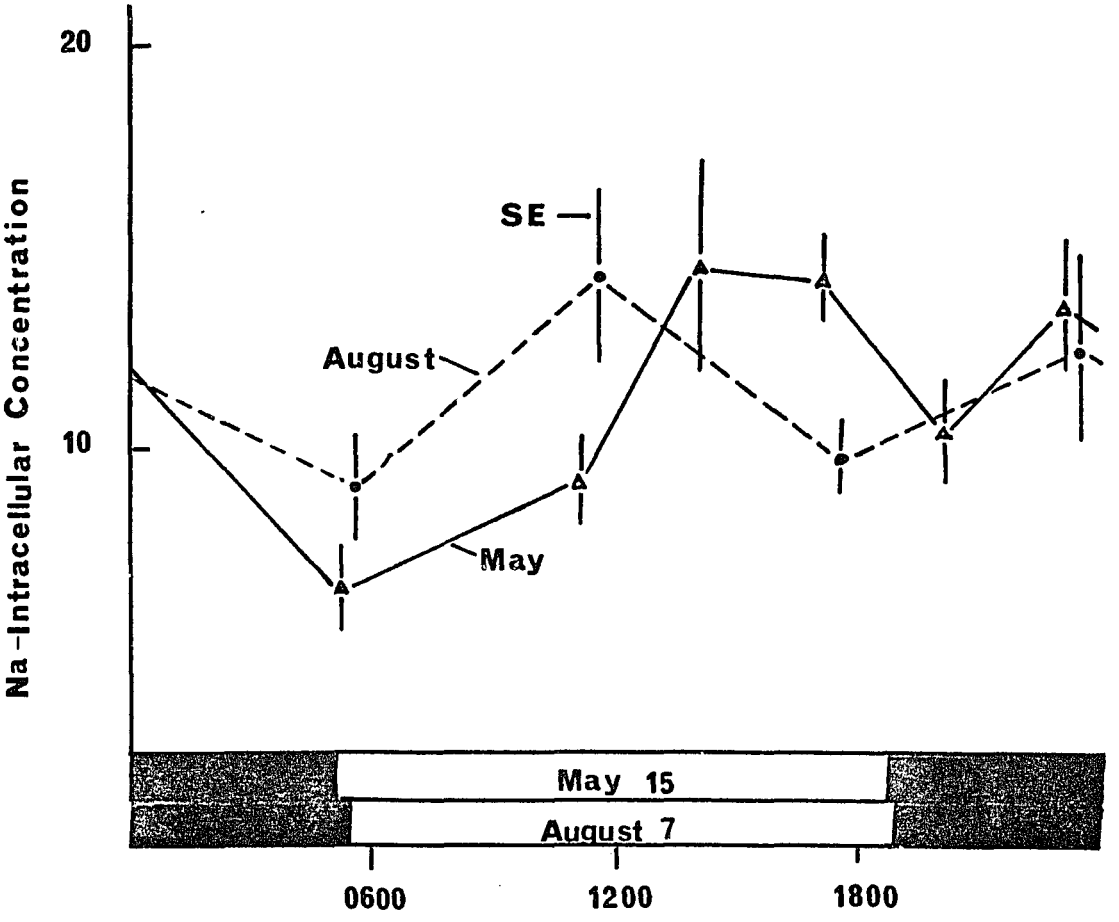

20
10
Na-Intracellular Concentration
SE
August
May
May 15
August 7
0600
1200
1800

Figure 6.

The diurnal rhythm of the extra/intracellular sodium concentration ratio in the brain of white-throated sparrows in mid-May and August ± S.E. The respective photoperiods are indicated on the abscissa.

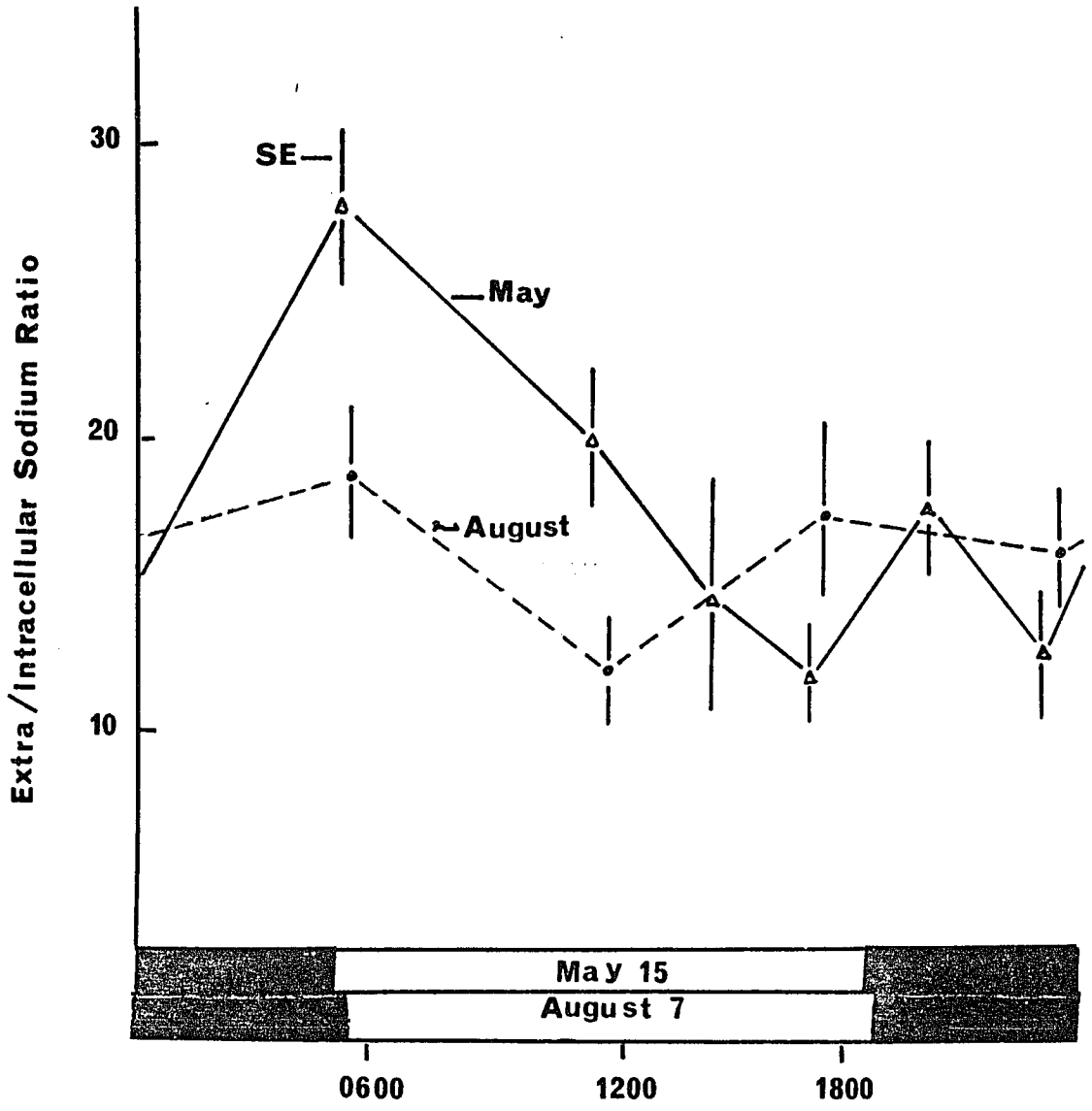
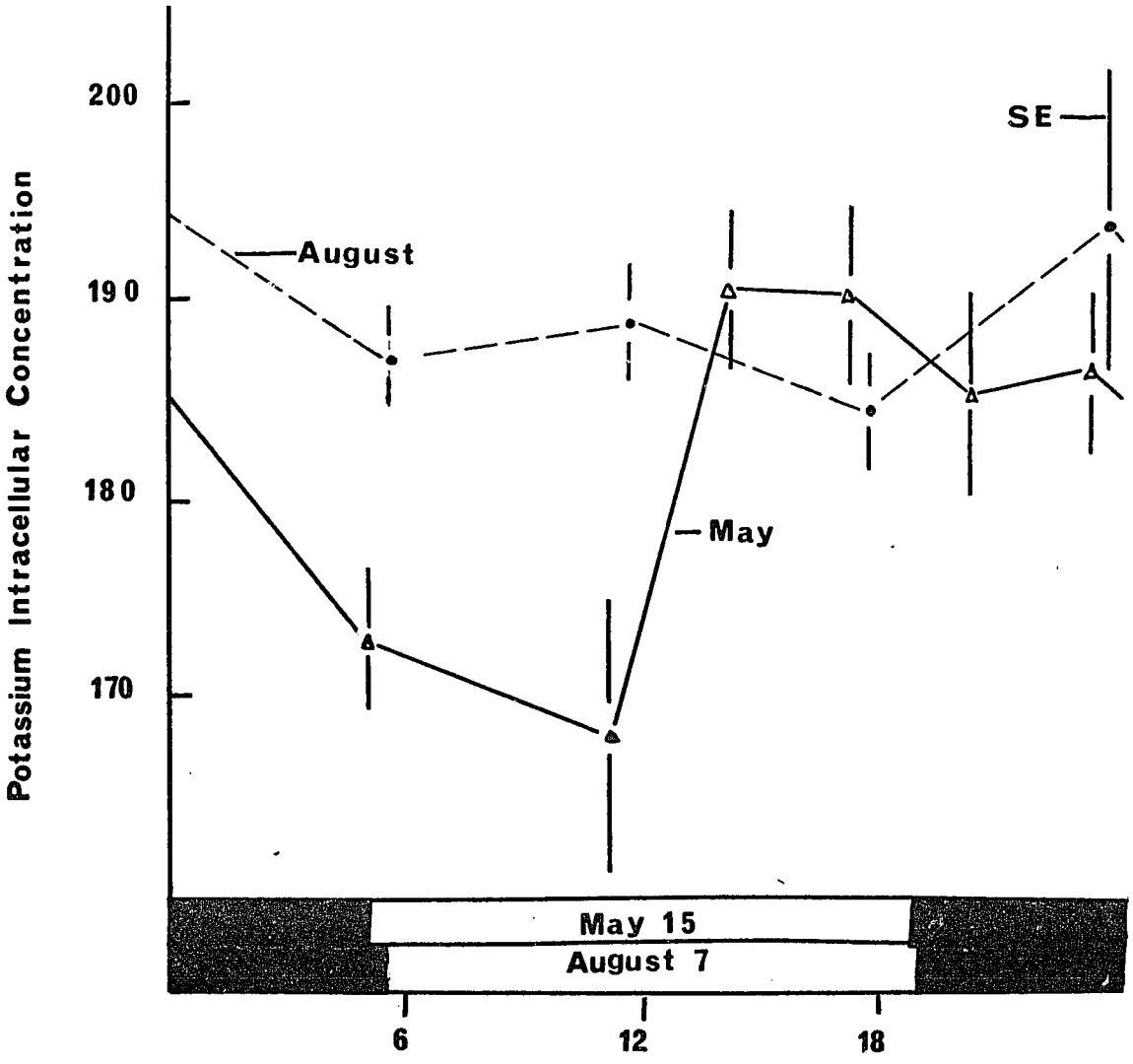

Extra /Intracellular Sodium Ratio
30
20
10
SE
May
August
May 15
August 7
0600
1200
1800

Figure 7.

The diurnal rhythm of intracellular potassium concentration in the brain of white-throated sparrows in mid-May and August. Values represent concentration as mEq/liter intracellular water + S.E. The respective photoperiods are indicated on the abscissa.


Potassium Intracellular Concentration
200
190
180
170
SE
August
May
May 15
August 7
6
12
18

Summary

Migratory fattening and nocturnal locomotor activity are indicies of physiological preparedness to migrate. In white-throated sparrows caged outdoors all of these characteristics are present in mid-May, and absent in April and August.

Diurnal plasma and brain levels of sodium, potassium, and chloride were measured in mid-May (spring migration) and August (postnuptial molt). Brain electrolyte content was also measured in April (prenuptial molt), and plasma potassium was measured in April and early May. The diurnal extracellular/intracellular concentration ratio of brain sodium (brain sodium ratio) was calculated for mid-May and August.

Similar diurnal electrolyte variations are present in April and mid-May. The diurnal electrolyte pattern in August is damped in all instances except plasma potassium, and the phase angle for peaks of plasma sodium and potassium, and for brain potassium is shifted from the pattern which occurs in mid-May. In April and mid-May a unimodal diurnal rhythm of brain sodium content is evident. A similar rhythm of brain potassium content occurs in mid-May. A rhythm of brain potassium content in April appears to be present but was

not statistically verified. In August no rhythm of brain sodium content exists and a bimodal rhythm of brain potassium content occurs. No rhythm of brain chloride occurs in mid-May or August, however, a peak of low amplitude occurs in April. In mid-May and August a diurnal rhythm of plasma sodium is present. In August the amplitude of the rhythm is lower and the time of the peak is shifted by eighteen hours. A diurnal rhythm of plasma potassium occurs in April, early May, mid-May, and August. The mid-May peak is also shifted from that in August by eighteen hours.

The brain sodium ratio is mid-May has a diurnal rhythm, but no rhythm occurs in August. Brain sodium ratio in mid-May is highest at sunrise, which is the time of day that locomotor activity is lowest. The brain sodium ratio falls during the day and remains low during the first part of the night when nocturnal locomotor activity occurs. Locomotor activity during the afternoon is low and brain sodium ratio is also low at that time. No correlation of brain sodium ratio with locomotor activity occurs in August.

No seasonal differences in brain sodium or chloride exist. Blood chloride and brain potassium were lower, and blood sodium was higher in mid-May than in any other season. There is no difference in the overall

seasonal means of intracellular sodium concentration or of the brain sodium ratio between mid-May and August.

Most seasonal comparisons when made using diurnal means indicate no quantitative difference. The most conspicuous seasonal differences of any of the measurements are in phase shifting and amplitude of the diurnal rhythms.

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Vita

Kenneth Bruce Davis, Jr. was born March 13, 1940, in Texarkana, Arkansas. He attended public schools in Texarkana graduating from the high school in 1958. He attended Texarkana Junior College for one year, and then entered the University of Arkansas from which he graduated in 1963 with the Bachelor of Arts in Natural Sciences. He entered the Graduate School of the University of Arkansas and received a Master of Science degree from the Department of Zoology in 1965. He was supported by a research assistantship awarded by the Arkansas Game and Fish Commission during his Master's work. In 1964 he entered the Graduate School of Louisiana State University, Baton Rouge, in the Department of Zoology and Physiology. For four years he served as a graduate teaching assistant, and held a research assistantship during the summer months. In 1967 he received a Federal Cost of Education Fellowship and during the fall semester of 1968 he served as an Instructor. In February of 1969 he accepted a position as Assistant Professor of Biology at Memphis State University. He was married in April of 1969 to Clara Ann Brister of Baton Rouge, Louisiana. He is now a candidate for the degree of Doctor of Philosophy.

EXAMINATION AND THESIS REPORT

Candidate: Kenneth Bruce Davis, Jr.

Major Field: Physiology

Title of Thesis: Seasonal and Diurnal Variations of Sodium, Potassium, and Chloride Levels in the Plasma and Brain of the Migratory White-Throated Sparrow, Zonotrichia Albicollis.

Approved:

Albert H. Meier
Major Professor and Chairman

May Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

Blanche Jackson

Date of Examination:

19 December 1969